Enantiomeric Resolution of Racemic Ibuprofen in Supercritical Carbon Dioxide Using a Chiral Resolving Agent

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ENANTIOMERIC RESOLUTION OF RACEMIC IBUPROFEN IN SUPERCRITICAL CARBON DIOXIDE USING A CHIRAL RESOLVING AGENT

Rebecca Valentine, Ph.D.

University of Pittsburgh, 2002

Given the inherent dangers associated with racemic pharmaceuticals, exhaustive investigations of techniques designed to separate enantiomers have been performed. Most methods are intrinsically expensive, consume vast quantities of organic solvent, and involve combinations of time consuming crystallization and/or chromatographic procedures. This dissertation reports herein the first step towards using pressure as a readily controllable variable during enantiomeric separation of racemic ibuprofen in liquid and supercritical carbon dioxide. Custom synthesized, CO_2 soluble and partially soluble resolving agents are added to the fluid phase to promote formation of diastereomeric salt pairs, which exhibit differences in their chemical and physical properties, such as solubility. Unlike enantiomers, which exhibit nearly identical solubility in carbon dioxide, separation of salt pairs may be accomplished by selective extraction at designated pressures due to the differences in their phase behavior in CO_2. Because formation of ion pair complexes occurs readily in media of low polarity, supercritical carbon dioxide offers an attractive alternative to traditional organic media.

DESCRIPTORS

Carbon Dioxide

Enantiomeric

Ibuprofen

Racemic

Resolving Agent

Separation

Supercritical

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1.0 INTRODUCTION

1.1 Chirality

In all biological systems homochirality is predominant and has been preserved since the beginning of evolutionary time. Homochirality refers to the spatial configuration of molecules, such as D- and L- amino acids, which are either produced by biological organisms or synthetically created. [(1)*] This spatial configuration is vital to biological activity because asymmetry dominates at the molecular level. From Pasteur's first studies involving biotransformations to Fisher's "lock – and –key" concept, our understanding of biomolecular interactions have grown, resulting in the development of highly specific pharmaceuticals.

By definition, a chiral material is one which lacks reflectional symmetry, i.e. exhibits a non-superimposable mirror image structure, and is termed as being "handed". The most common chiral compounds which exist are enantiomers. These materials are typically characterized by an asymmetric, tetrahedral carbon atom located at the center of the molecule. These molecules can exist as stable, observable stereoisomers if their energy barrier of conversion exceeds 80 KJ/mole. In addition, compounds which exist as enantiomers have nearly identical physical and chemical properties in an achiral environment, making their resolution into individual components challenging.

*Parenthetical references placed superior to the line of text refer to the bibliography

When these enantiomers are present in equimolar amounts within a mixture, the resultant mixture is termed racemic. These preparations are optically inactive, because the net rotation of plane polarized light is negated by the equal concentrations of each enantiomer. The first successful attempt to resolve enantiomers from their racemic mixture was performed by Louis Pasteur, in which he manually resolved a racemic mixture of sodium ammonium tartrate into its individual enantiomers. [2]

Diastereoisomers are non mirror image stereoisomers that possess more than one asymmetric center. Unlike enantiomers, diastereomers may be individually isolated because differences exist in their physical and chemical properties, such solubility and melting point. [2] Enantiomers may be transformed into diastereomers by either covalently or non-covalently coupling the enantiomers of a racemic mixture to another chiral molecule possessing at least one asymmetric center. This methodology defines a separation route by which two previously inseparable materials may be isolated by conventional techniques.

1.2 Pharmaceutical Trends

The importance of determining the pharmacological activity of each component in a drug has now gained full acceptance as shown by the substantial number of single isomer pharmaceuticals entering the commercial market. The motivation for this single isomer trend has been provided in part by the FDA and in part by the production of a host of pharmaceuticals previously protected by 17 – 20 year patent production laws. Concerns which the FDA are requiring pharmaceutical producers to address include:

- pharmacological properties of the individual enantiomers and of the racemic mixture
- assays which determine enantiomeric purity
- the need to produce as a single isomer
- economic incentives to develop separation methods for existing racemic mixtures

Those particular chiral drugs, whose patents are expiring, are attracting a multitude of oversea producers. This would provide pricing competition and increase the "generic brand" availability from producers with large scale capacities. [3]

Chirally active drugs also represent a large share of the pharmaceutical market. In 1996 chiral drugs composed $73 billion dollars of the market, which was an 18.6% increase from the previous year. Chiral bulk active drugs used in producing dosage forms were valued at $13.5 billion dollars. Technology Catalysts International has estimated that by the year 2000, dosage formulations will exceed $90 billion. [3] Table 1 outlines the sales figures and predicted growth rates for the three top categories of drugs produced in the United States.

There does exist a small minority of these drugs, which are eligible for a racemic switch as shown in Figure 1. A racemic switch is a reprocess and reformulation of a racemic mixture, previously approved by the FDA, into its single isomer product. As shown in Table 2, a host of common name brand pharmaceuticals are eligible or are currently undergoing a racemic switch. It is important to note the number of candidates within the anti-inflammatory and analgesic category.

Table 1 Sales Figures and Predicted Growth Rates [3]

Chiral drugs are $73 billion global market

	Sales			
		Dosage Form ($ millions)		
	Bulk active 1996 ($ millions)	1996	2000	Predicted average annual growth rate
Category				
Antibiotics	$ 4,880	$ 19,527	$ 20,905	1.7%
Cardiovascular	2,040	17,530	19,535	2.7
Hormones-Endocrinology	1,340	8,006	11,950	10.5
Cancer	1,310	7,547	8,390	2.7
Hematology	1,025	5,199	5,050	-0.7
Central Nervous System	715	4,065	7,505	16.6
Vaccines	730	3,970	4,550	-3.5
Antivirals	300	1,815	4,420	25.0
Organ Rejection	295	1,475	1,650	2.8
Respiratory	244	1,201	1,240	0.8
Analgesics	355	895	815	-2.3
Ophthalmic	125	675	630	-1.7
Benign Prostate Hyperplasia	77	450	750	13.6
Dermatology	46	545	675	5.5
Anesthesia	0	0	60	na
Other	0	0	2,000	na
Total	**$ 13,482**	**$ 72,900**	**$ 90,025**	**5.4%**

Table reproduced from S.C. Stinson [3]

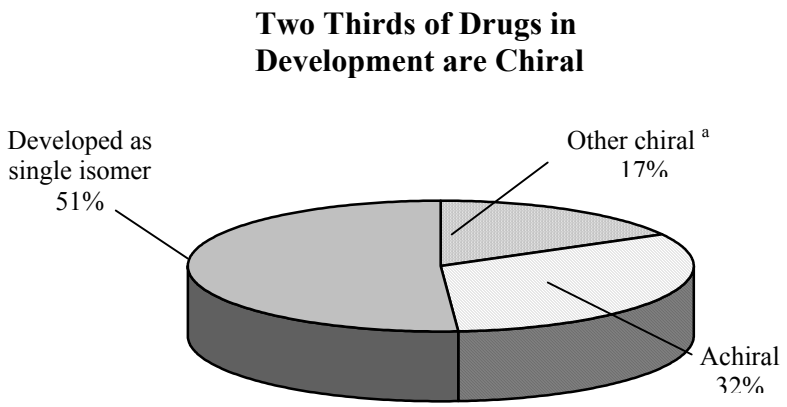


Figure 1 Drug Development Distribution (3)

There exists a multitude of methods and techniques specifically designed for enantiomeric separations, though not all methods are equally applicable for every racemic mixture. Drug development within the pharmaceutical industry focuses heavily on asymmetric synthesis, enzymatic resolution, crystallization techniques, chromatographic and membrane processes, and combinatorial chemistry. The common denominator in all of these processes is that these are organic media based methods. This research project is an attempt to develop an organic media free separation process, while drawing heavily from the practices and principles of the traditional separation methods.

Table 2 Candidates for Racemic Switches [4]

Cardiovascular		***Anti-inflammatory and analgesic***	
Acebutolol	Alprenolol	Cicloprofen	Corticosteroids
Atenolol	Betaxolol	Dihydroxythebaine	Feribufen
Bisoprolol	Bopindolol	Fenoprofen	Flurbiprofen
Bucumolol	Bufetolol	Ibuprofen	Indoprofen
Bufuralol	Bunitrolol	Ketoprofen	Minoxiprofen
Bupranolol	Butofilolol	Pirprofen	Suprofen
Carazolol	Carvedilol	Triamcinolone	
Disopyramide	Dobutamine		
Indenolol	Mepindolol	***Anticancer***	
Meupranolol	Metoprolol	Cytarabine	
Nadolol	Nicardipine		
Oxprenolol	Pindolol	***Antibitoics, anti-infectives, and anti-virals***	
Propranolol	Sotalol	Ciprofloxacin	Norfloxacin
Toliprolol	Verapamil	Ofloxacin	
Xibenolol			
Central Nervous System		***Hormones and genitourinary***	
Dobutamine	Fluxetine	Benzyl glutamate	Butoconazole
Ketamine	Lorazepam	Calcitonin	Estradiol
Meclizine	Oxaprotiline	Fluorogestone	Gonadorelin
Phenylpropanolamine	Thioridazine	Norgestrel	Testosterone
Polychloramphetamine	Tomoxetine		
Toloxatone	Viloxazine	***Antihistamines and cough-cold***	
		Astemizole	Terfenadine
Respiratory			
Albuterol	Metaproterenol		
Terbutaline			

Source: Technology Catalysts International

Table reproduced from S. Ahuja [4]

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 Ibuprofen as a Model Compound

In the anti-inflammatory class of pharmaceuticals, ibuprofen is an interesting example of one which is still sold as a racemic mixture. The single isomer product has a greater commercial value than the racemic mixture, but production has been limited by legal and process difficulties. In addition, ibuprofen is the one member from the NSAID family which has proven to be the most challenging to enantiomerically purify. A large research investment has been made in order to develop techniques specific to ibuprofen. Out of this research has come information used to develop methods from simple crystallizations to complex membrane separations.

For this research project, ibuprofen was chosen as a model compound for the following reasons;

- A wealth of information concerning its physical and chemical properties, reactivity, and production is readily available.
- It is a simple and stable molecule as shown below in Figure 2.
- Development of new separation methods for this and related molecules is still a large area of pharmaceutical research.
- Recent attempts have been made to enantiomerically separate ibuprofen in supercritical carbon dioxide.

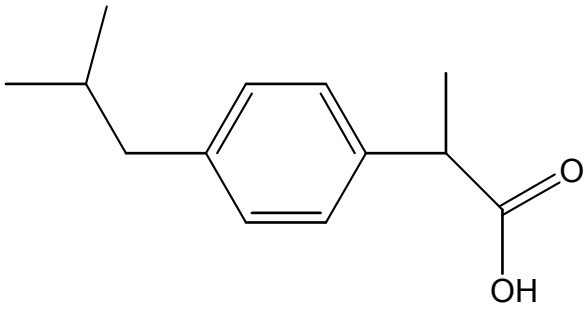


Figure 2 Chemical Structure of Ibuprofen

2.2 Chromatographic Separation Methods

Presently, there are a variety of resolution techniques by which the enantiomers of ibuprofen are separated. Each method introduces an asymmetric environment either intramolecularly, in which a diastereomeric pair is chemically produced to interact with a nonchiral medium, or intermolecularly, where the underivatized free acid interacts with a chiral medium. [5] For each method, several advantages and disadvantages prevail depending upon factors such as time, purity, chemical processing, and inherent side reactions. These factors have all contributed to the lack of a universal enantiomeric separation regime for racemic ibuprofen.

The most common method to date for the enantiomeric resolution of ibuprofen enantiomers and, in general chiral materials, is high performance liquid chromatography. Construction of a chiral stationary phase or pre-derivatization of the individual enantiomers to produce the diastereomeric pair, are the two important techniques that are employed. Though these techniques vary from normal to reverse phase and utilize a variety of detector systems, the separations achieved thus far are similar.

There are extensive reports in the literature concerning the pre-derivatization technique. [7,8,9,10,11,12,13] In most instances ethyl chloroformate is used to couple a chiral ligand to the racemic enantiomers in order to produce the diastereoisomeric pair. A separation factor, which is defined as the ratio of the time required for the last enantiomer to elute divided by the first enantiomer to elute, is used to evaluate the performance of the technique. Separation factors of 1.09 to 1.12 for ibuprofen were reported, thus yielding an acceptable separation. (In the literature, a separation factor in excess of 1.2 is desirable.) Though the enantiomers of ibuprofen were resolved in under 20 minutes, there are inherent drawbacks to this method. These drawbacks include:

- The rates of formation for the diastereoisomer pair may be different, leading to an incorrect ratio of the two enantiomers.
- Impurities within the reaction mixture could lead to a multiple number of diastereoisomeric pairs.
- Racemization may occur and its extent is dependent upon the coupling agent and reaction solvent.
- A chemical, covalent bond is formed between the coupling agent and ibuprofen, which is difficult to disassociate to recover the free acid of ibuprofen. [9]

The deficiencies in the pre-derivatization method prompted investigators to develop a technique by which ibuprofen would remain chemically unmodified. Instead, a column which possessed a chiral packing material was employed. [14,15,16,17,18,19] These chiral packing materials most often are composed of derivatives of cellulose, cyclodextrins, proteins, and amino acids. [14] Base line resolution of enantiomeric ibuprofen has been successful in under 30 minutes. The separation factors, though, exhibited a column dependent broad range from 1.08 for the cyclodextrin based columns, 1.06 for the Pirkle silica gel modified columns, and a high of 1.29

for modified ovomucoid columns. The disadvantage of this method is that there appears to be no universal chiral column and analysis conditions. In addition, large volumes of organic based mobile phase are required for component elution, and this volume of solvent must be evaporated in order to recover the free acid of ibuprofen. Obtaining ibuprofen with the minimal solvent contamination as required by the FDA becomes more costly as the amount of ibuprofen processed increases. This method, however, is still preferred to pre-derivatization since the number and availablility of different chiral columns commercially available has increased.

2.3 Other Methods

Another method of producing enantiomerically pure ibuprofen is selective enzymatic esterification using lipases. Taking advantage of the naturally inherent selectivity of the enzyme's active site, reactive discrimination occurs between the enantiomers in the racemic mixture. Though not exclusive, one enantiomer in general "fits" better in the active site and is therefore converted to its corresponding ester at a higher rate. [20] Rantakyla and Aaltonen esterified racemic ibuprofen with n-propanol in supercritical carbon dioxide by means of an immobilized lipase. The resulting enantiomeric excess was approximately 70% of the S(+) enantiomer with 15 to 20 % conversion. [6] This process is dependent upon a number of variables including optimum temperature, ibuprofen and alcohol concentration, pressure, water content, and enzymatic lifetime.

The advantage of this method is the high enantiomeric excess, resulting from the inherent selectivity of the enzyme. The enzyme is reusable and the products from the reaction are easy to

separate. The disadvantages of this method are related to the number of system parameters which must be optimized for the enzyme, and the selectivity of the system is limited by the extent of conversion. [21]

Gas chromatography, mass spectrometry, electrochromatography, capillary electrophoresis, and nuclear magnetic resonance have also been utilized as potential analytical techniques in enantiomeric separations of ibuprofen. [22,23,24,25,26] These methods have proven of less utility due the inability to achieve baseline resolutions or inability to recover the free acid of ibuprofen after separation. In addition, the effectiveness of high performance liquid chromatography has masked advanced development of these methods.

A recent development in the separation of ibuprofen enantiomers has been in supercritical fluid chromatography. [27,28,29,30] Ibuprofen has been shown to exhibit solubility in supercritical carbon dioxide at optimum conditions of 70°C and a supercritical carbon dioxide density of 0.7 g/ml. [29] SFC behaves similar to normal phase high pressure liquid chromatography, but can be run at 3 to 10 times faster than normal phase HPLC. In addition, SFC does not produce problems of column deactivation, long equilibration times, and leaching of a chiral constituent. [27] SFC, when performed with carbon dioxide, also provides an environmentally sound separation technique.

SFC utilizes a chiral column and a mobile phase, which usually consists of a combination of carbon dioxide and a polar modifier such as methanol or tetrahydrofuran. A separation factor for ibuprofen of 1.14 was obtained with baseline resolution. [27] One advantage that traditional HPLC exhibits over SFC is peak shape and resolution. Separations in SFC tend to produce peaks in which peak tailing is evident. This is thought to be attributed to hydrogen bonding interactions with the chiral packing of the column. [28] Despite this disadvantage, SFC is attractive due to the

use of a benign mobile phase and the speed at which separations may be performed. Unfortunately, this technique has not been well received as a prep scale separation method.

2.4 Physical Separation Methods

All of the previous separation techniques utilize a chromatographic methodology in some aspect. The instrumentation and processing would be expensive on an industrial scale, and the need for a cheap and reliable method is evident. Crystallization has been the predominant separation technique to resolve an enantiomeric mixture into its individual isomers on the industrial scale. There are three primary methods of crystallization for enantiomeric resolution. These include:

- Preferential crystallization – stereospecific growth of each individual isomer in two different crystallizers from solution. This process requires no resolving agent.
- Diastereomeric crystallization – resolving agent binds to enantiomers to form a diastereomeric salt pair. These salts are separated as a function of their phase behavior.
- Catalytic kinetic resolution – resolving agent reacts at different rates with each enantiomer. [31]

Diastereomeric crystallization has dominated as the technique of choice among industrial pharmaceutical companies for the resolution of ibuprofen enantiomers. [31,32,33,34] A common resolving agent used for the enantiomeric separation of ibuprofen is L-lysine. It has been found that upon salt formation, the D-ibuprofen-L-lysinate salt exhibits about two thirds of the solubility

of the L-ibuprofen-L-lysinate salt. [31] This indicates that a decent separation of the L-ibuprofen-L-lysinate from the D-ibuprofen-L-lysinate salt may be readily achieved. The formation of an optically active salt is also ideally desirable, since the salt is more soluble in a water medium than the corresponding free acid of ibuprofen. [32] Pharmacological studies have proven that the amino acid salt of ibuprofen exhibits increased adsorption in the blood stream as compared to the free acid. [35] Thus, an effective, inexpensive, and pharmacologically valuable product is produced by this technique.

2.5 Supercritical Fluids

Though researchers have studied supercritical fluids for more than one hundred years, the realization of its solvent potential and utilization has been evident only for the last two decades. [36] A fluid is considered supercritical when the temperature and pressure combination exceed those of its critical point. These media possess unique properties, which make them attractive for many chemical processes. The prominent feature of a supercritical fluid is the ability to adjust the solvent strength by varying the density. This may be accomplished without changing the bulk composition or reaction temperature. [38] In addition to its variable solvent strength, other properties add to its attractiveness as a chemical solvent. It exhibits liquid like densities, while maintaining gas like diffusivities and viscosities. Low surface tension allows SCF's to penetrate into microporous or geometrically complex matrices. [36] Solvent removal may be accomplished by simply reducing the pressure, thereby creating a clean and safe alternative to traditional solvent

mediums. Currently, these fluids are used commercially in such processes as coffee decaffeination, edible oils extraction, isomer separation, and in the treatment of waste streams. [38]

The most commonly used supercritical fluid is carbon dioxide. Supercritical CO_2 possesses reasonable critical properties (T_c= 31.1°C and P_c= 72.8 atm), chemical inertness, and low toxicity. [39] The dielectric constant, which is a description of its "tunable" solvent strength ranges from 1.05 to 1.5 at 60°C and 50 to 500 bar, respectively. This small range allows for fine tune control of pressure sensitive processes. Supercritical CO_2 also possesses induced dipole moments, Lewis acid properties, and quadrapole interactions. These characteristics allow CO_2 to solvate a number of chemical compounds with varying polarity.

For many compounds, these interactions are not adequate to efficiently partition the sample into the bulk supercritical fluid phase. Other supercritical fluids may overcome this deficiency by a change of the physical properties in the bulk medium, such as fluoroform or sulfur hexafluoride, but reasonable critical properties, cost, and environmental concerns, such as the greenhouse effect and ozone layer degradation, may limit their use. [38] The physical properties of some supercritical fluids are shown in Table 3.

Table 3 Critical Temperatures and Pressures for Supercritical Fluids [36]

SOLVENTS	CRITICAL TEMPERATURE (^{O}C)	CRITICAL PRESSURE (BAR)
Water	374.2	220.5
Carbon Dioxide	31.1	73.8
Ethane	32.2	48.8
Ethylene	9.3	50.4
Propane	96.7	42.5
Propylene	91.9	46.2
Cyclohexane	280.3	40.7
Isopropanol	235.2	47.6
Benzene	289.0	48.9
Toluene	318.6	41.1
p – Xylene	343.1	35.2
Chlorotrifluoromethane	28.9	39.2
Trichlorofluoromethane	198.1	44.1
Ammonia	132.5	112.8

3.0 RESEARCH OBJECTIVES

Direct resolution of ibuprofen enantiomers as a function of their diastereomeric derivatives is a common method that is employed in both chromatographic and crystallization methods. Diastereomeric crystallization is classified as a "low technology" approach due to its simplicity, yet more than 65% of market drugs are resolved by this method. [4] The advantage of using a resolving agent in solution is the short reaction times based upon the bonding characteristic of the resultant complexes such as diastereomeric ion pairing, hydrogen bonding, or hydrogen ion pairing. [30] In addition, the original compounds, namely the resolving agent and the free acid of ibuprofen, may be recovered through simple cracking techniques.

Our goal is to develop a separation system, which enables us to resolve the enantiomers of ibuprofen in the form of their diastereomeric salts. This process will be designed to operate in a thermodynamic resolution mode, which is a proven technology in organic solution and thus, potentially transferable to carbon dioxide. Racemic ibuprofen is soluble in CO_2 at 40 ^{o}C as shown from experimental phase behavior measurements, which were consistent with the results of Khundker and coworkers. [29] The solubility of ibuprofen alone in carbon dioxide indicates that a feasible separation process may be designed.

Fogassy and Simandi have attempted to resolve the enantiomers of ibuprofen as diastereomeric salts in carbon dioxide. They formed ibuprofenate salts in organic media such as chloroform prior to loading a high pressure reactor. The unreacted free ibuprofen was then extracted with carbon dioxide, assuming that the ibuprofenate salts were insoluble. Enantiomeric excesses up to 42% were achieved. [43] The limitation to this process is based not only on the selectivity of the chiral amine used for salt formation, but also the solvent in which

salt formation took place. The investigators were not taking advantage of the solubility difference between the diastereomeric salt pair. Instead they are relying on the selectivity of an organic chiral amine, which in general does not possess the magnitude of selectivity of something like an enzyme. In addition, this selectivity may change as a function of solvent characteristic.

Specifically, we would like to propose a somewhat different approach than that of Fogassy et al., namely the development of a carbon dioxide soluble chiral resolving agent for diastereomeric extraction of the desired enantiomers. As opposed to Fogassy who prepared their diastereomeric salts in organic solution previous to CO_2 extraction, our separation scheme involves a combination of *in situ* salt formation and extraction. This will be accomplished by creating custom designed, CO_2 soluble resolving agents which will bind specifically to the enantiomers of ibuprofen. Any observed differences in solubility between the salt pair will be maximized as a function of the operating pressure, thus, taking advantage of the variable solvent strength of the supercritical carbon dioxide medium.

4.0 MOLECULAR DESIGN OF A CHIRAL RESOLVING AGENT FOR THE ENANTIOMERIC RESOLUTION OF RACEMIC IBUPROFEN

4.1 Introduction

The application of computer based simulation models to describe the physical properties of chemical compounds has become a powerful tool in the fields of biochemistry, organic chemistry, and chemical engineering. These computer models utilize a force field, which contains a set of analytical potential energy functions operating within the realm of classical mechanics. These force fields are used to calculate molecular energy minimizations and dynamics to provide information relating to structural, physical, and electrical properties of a particular compound.

There exists many common force fields with which to perform these calculations, such as Allinger's MM2, Allinger's modified MM3, Amber and CHARM, and OPLS. [44,45,46,47] The principle function of each force field is to mathematically describe and translate a multitude of physical phenomena such as intrinsic strain of organic molecules, structure and dynamics of simple liquids and solids, thermodynamics of protein binding, receptor ligand interactions, and transitional conformations into understandable, representative pictures of chemical processes. [44] Due to an increased understanding of the complexities of these chemical and biological processes, the number of modified and new force fields have been increasing in the past ten years to effectively incorporate uncommon geometry and polar properties. [45] In addition, many of these computational methods have been modified for use in supercritical fluid technology, especially in extraction applications. [48,49,50]

4.2 Design Rational

4.2.1 Selection of a Resolving Agent and CO_2 Philic Tail

The primary drawback to using supercritical carbon dioxide as a process media for enantiomeric separation is its poor ability to solubilize most polar compounds in at moderate pressures. Fortunately, racemic ibuprofen is soluble in carbon dioxide, which has also been confirmed by Khunder, et.al. [29] The challenge, then, is to develop appropriate, highly CO_2 soluble (or "CO_2 philic") resolving agents that not only exhibit good CO_2 solubility, but also maintain selectivity similar to that of its native (CO_2 phobic) state, taking into consideration variable solvent effects. The first step in this process is to identify chiral bases that have been used successfully in ion pairing separation schemes for racemic acids. A listing of these agents and their potential carbon dioxide solubility is shown in Table 4.

Two important chemical criteria must be applied to potential agents in order to render them suitable for enantiomeric resolution in a carbon dioxide environment. First, the reaction conditions used during the derivatization process must be mild enough so as not to induce inversion of the chiral center, thus altering the agent's natural selectivity. The second criteria focuses on the structure of the natural agent itself. Though there does not exist a well defined set of rules for enantioselectivity of an ion pairing agent, empirical evidence suggests that functional groups, particularly those that possess hydrogen bonding potential, located close to the chiral center are important in defining a rigid, steric environment for selectivity to be determined. [30] Modification of those groups may result in a loss of the agent's natural selectivity. Thus, a resolving agent should be derivatized at a functional group far removed from the chiral center.

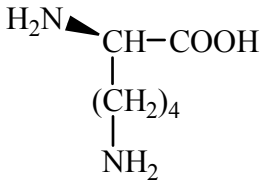
From the list of potential candidates in Table 4, L-lysine and quinine were chosen based on the previous requirements for chemical modification. The native chemical structures of these two resolving agents are shown below in Figure 3.

Table 4 Chiral Bases Utilized in the Resolution of Racemic Acids [51,52]

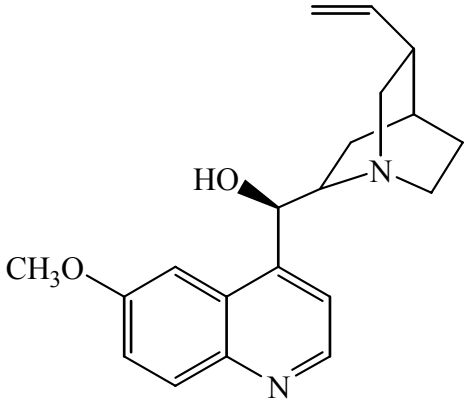
CHIRAL BASE	CO_2 SOLUBILITY
L-lysine	* Negligible
Aspartame	* Negligible
Cinhonine	* Negligible
Quinine	** Partial
α–Methylbenzylamine	** Partial
α–Methyl DOPA	** Partial
Troger's Base	* Negligible
1R, 2S-Ephedrine	** Partial
S-(+)-Phenylglycinol	** Partial
S(-)-2-Amino-3-phenyl-1-propanol	** Partial

* Negligible = 10^{-8} mole fraction at 2000 bar

** Partial = estimated as less than 20 weight % soluble at 35 ^{o}C and 7000 psi



L-Lysine



Quinine

Figure 3 Native Structures of L-Lysine and Quinine

CO_2 philic ligands may be classified into three primary groups, based upon their chemical compositions as shown in Table 5. For our experimental purposes we have chosen to derivatize our agents with fluoroalkyl, fluoroether, and silicone tails. These materials are readily available and relatively easy synthetic preparations are known. The chemical structures of the proposed CO_2 philic agents are shown below in Figures 4a thru 4c.

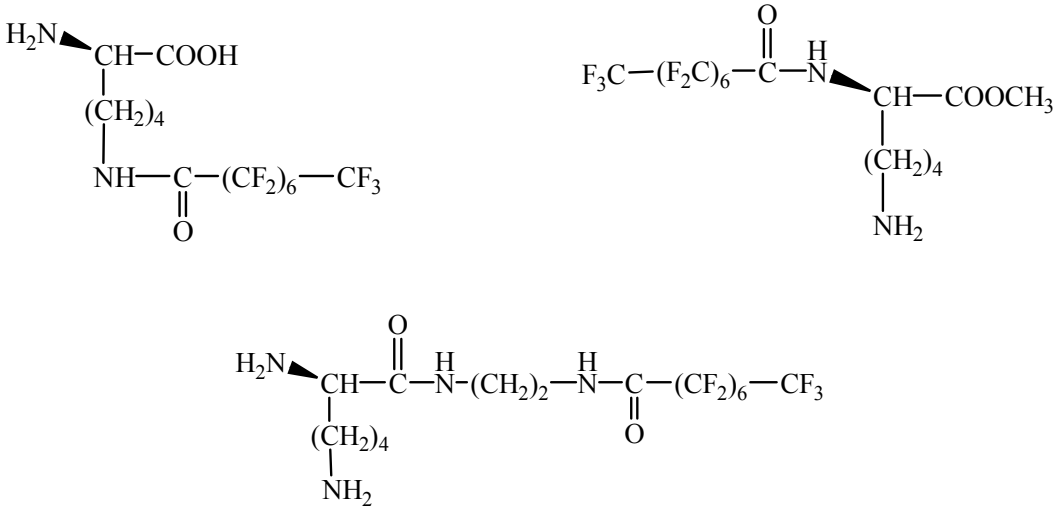


Figure 4a L-Lysine Fluoroalkyl Analogs

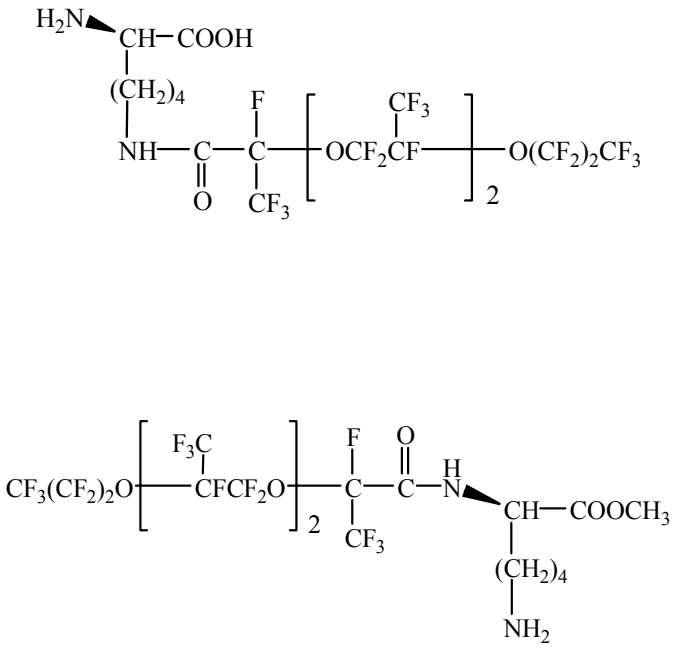
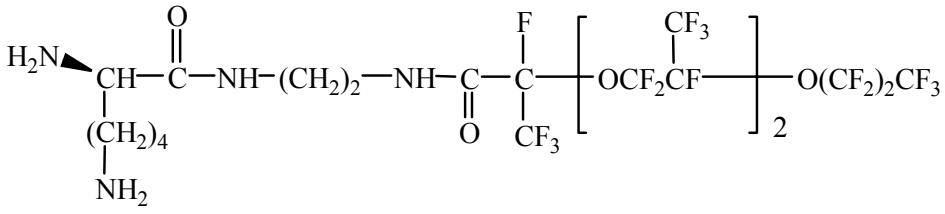
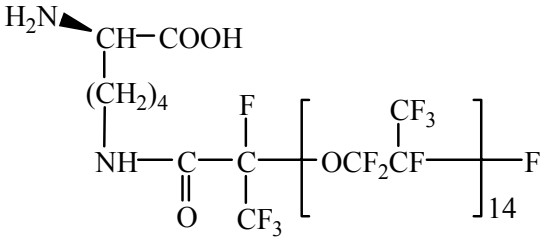
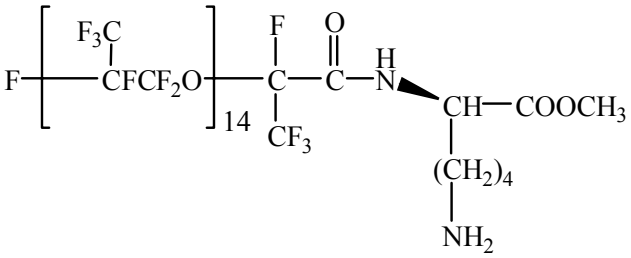


Figure 4b L-Lysine Hexafluoropropylene Oxide Analogs







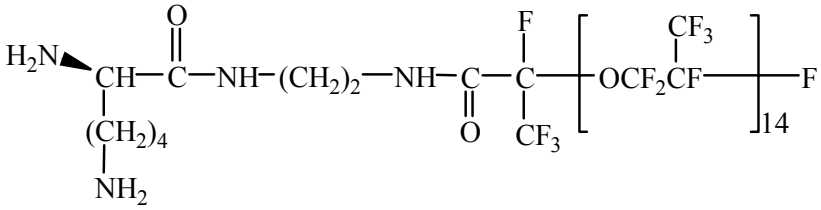


Figure 4b L-Lysine Hexafluoropropylene Oxide Analogs Cont.

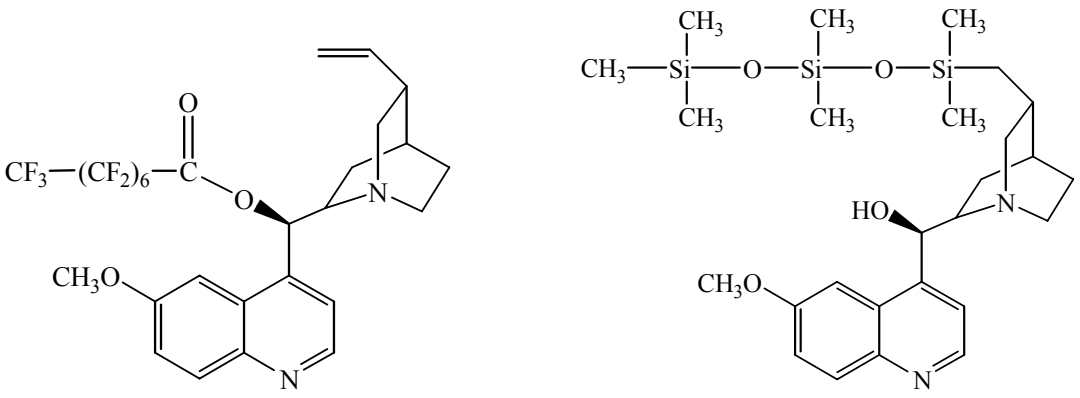


Figure 4c Quinine Fluoroalkyl and Silicone Analogs

Table 5 Liquid and Supercritical CO_2 Philic Functional Groups

FUNCTIONAL GROUP	REASON
Tertiary amines, aliphatic esters and ethers	• Lewis Base
Fluoroalkyl and fluoroethers	• Low solubility parameter • Low cohesive energy density
Silicones	• Low solubility parameter

4.2.2 Calculation Design

Several resolving agent candidates were screened for ibuprofen using computational chemistry. Predicting the selectivity of a chiral resolving agent was estimated using a molecular mechanics package to evaluate the lowest energy conformations of the diastereomeric salt pairs composed of ibuprofen and a particular resolving agent. Molecular mechanics is an empirically based calculational design whose fundamental, physical premise is that all bond lengths between

atoms have a natural length and angle which defines the minimum energy of the molecule. [53] Theoretical, structural differences between these molecules suggests differences in physical properties which can then be tested experimentally.

The total energy of a molecular conformation compared to that of a hypothetical strain-free molecule of the same constitution is termed the steric energy. [54] Steric energy is the parameter which will be used to evaluate the selectivity of a resolving agent. The differences in steric energy between the ibuprofenate diastereomeric salts gives an indication of how structurally different the two molecules are. The greater the difference in steric energy between the salt pairs, the greater the three dimensional structural dissimilarity, and, thus, variances in their physical properties.

A minimum difference of 3 kcal/mol for any diastereomeric pair was required for a resolving agent to be classified as selective. This standard is derived from the relationship between stability and isomeric composition at equilibrium, which may be simply expressed in terms of the equilibrium constant K_{eq} as shown below;

$$K_{eq} = \exp(-\Delta E/RT) \qquad (4\text{-}1)$$

where ΔE represents the energy difference between the two isomers, T is the absolute temperature in Kelvin, and R the gas constant. Table 6 lists the ΔE required to obtain specific enantiomeric compositions. From Table 6 an energy difference of 3 kcal/mol would yield an enantiomeric excess greater than 99%. [54]

Table 6 Theoretical Enantiomeric Compositions

MORE STABLE ISOMER (%)	LESS STABLE ISOMER (%)	ENERGY DIFFERENCE ΔE (KCAL/MOL)
50	50	0
75	25	0.651
90	10	1.302
95	5	1.744
99	1	2.722
99.9	0.1	4.092

4.3 Experimental

The relative steric energy difference between the minimized conformations of the various salts with modified and unmodified resolving agents as reported in Tables 7a – 7d was calculated. The specific interactions between the resolving agents and ibuprofen were based upon relevant crystallographic data. [55,56,57,58,59,60] The models were created in the editor program of the CAChe molecular modeling software. From the general structure, a mechanics calculation based on Allinger's MM2 force field was performed in order to obtain local minimized structures. To confirm that a minimized structure had been determined from the mechanics calculation, a dynamics calculation was run in order to verify the global energy minimum conformation. In this computation the molecule is theoretically heated from 0 to 600 Kelvin. A trajectory plot of potential energy versus time is found. From this plot, ten random structures were chosen and their conformations re-minimized. The difference in the steric

energy values for the ten random structures was an indication of the stability and energy minimum of the final conformation.

4.4 Results and Discussion

Molecular mechanics was used as a measure for conformational analysis and not as a predictor of solubility or phase behavior in a CO_2 solution. The modeling employed here simulates a single molecule in vacuo, and thus the molecule is not a solid state structure and does not include packing forces. Weak bonds may exists in the solid state, such as ionic or hydrogen bonds, that may or may not exist in solution. These types of bonds are more likely to exist in media of low polarity such as hydrocarbons and carbon dioxide, and less likely in media of higher polarity such as water or other protic solvents. [61] Since the proposed processing medium is supercritical carbon dioxide, which exhibits a Hildebrand solubility parameter similar to perfluoro hexane, it is assumed that those weak bonds formed from the chiral complex are stable within this fluid.

4.4.1 Effect of a Fluoroalkyl Tail on the Selectivity of L-Lysine

As shown in Table 7a, free L-lysine exhibits a high selectivity towards the enantiomers of ibuprofen when binding occurs at the ε amine. Preferential complexation at that basic site is again verified by experimental crystallographic data. [55,56,57,58,59,60] This amino acid is currently

the resolving agent of choice for resolution of racemic ibuprofen by crystallization techniques which employ chiral resolving agents. [31] Upon chemical modification with an eight carbon, fluoroalkyl tail, the selectivity of the amino acid is predicted to decrease. Functionalization at the α amine produced a molecule which exhibits a slight decrease in selectivity. Since the ε amine functions as the site at which ion pairing occurs, a slight predicted decrease in the selectivity of the agent is reasonable. It can be proposed that the tail itself is the limiting factor. The ε amine is located far enough away from the tail in structure 2 so as not to be effected by the electron withdrawing effect of the fluorinated chain. The physical presence of the tail itself may be interfering in the formation of the rigid environment required for chiral discrimination.

A significant reduction in the selectivity of the agent derivatized at the ε amine (structure 3, Table 7a) is observed as compared to its corresponding modified structure where the fluoro tail is placed at the α amine (structure 2, Table 7a). In this instance the same additional hydrogen bonding capability is provided by the presence of an amide located at the tail section. The difference, then, is the complexation at the α and ε amines. As shown in structure 1, the ε amine is the favored site. Therefore, it is expected that complexation at the α amine is disfavored, and this result is consistent with the results obtained for the native molecule (structure 1). The fluoroalkyl tail, then, does not significantly appear to effect chiral discrimination.

The last agent in Table 7a was created in order to observe the effect of leaving both primary amines free for ion pairing with ibuprofen's carboxy group. In addition, a two carbon spacer was added between the native amino acid and the fluoroalkyl tail, in order to shield the α amine from the highly electron withdrawing effect of the fluorinated tail. The effect of this structural configuration is interesting, in that the selectivity at the α amine binding site is

predicted to increase as compared to its binding at the α amine of the native molecule(structure 1 in Table 7a). The additional, potential hydrogen bonding capability contributed by the amide group located in close proximity to the α amine may possibly contribute to the stabilization of the chiral complex. A significant decrease in chiral discrimination is observed with complexation occurring at the ε amine. Due to the length of the tail and its flexibility about its single bonds, the ability of ibuprofen, a relatively bulky substrate, to preferentially bind at the ε amine may be substantially minimized.

4.4.2 Effect of Krytox Perfluoropolyether Based Tail on the Selectivity of L-Lysine

The results for L-lysine modified with a perfluoropolyether tail are shown in Table 7b. Here, we have used the structure for a Dupont product (Krytox functional fluids), which is an oligomer of hexafluoropropylene oxide. Although Krytox fluids are available in several molecular weights, we have confined our modeling work to that with an average molecular weight of 2500. This particular material was chosen due to its previous use in our laboratories as a CO_2 soluble modifying agent. [62,63,64,65,66] For the agents shown as structures 1 and 2 in Table 7b, the selectivity as compared to the native agent was significantly reduced. The difference in steric energies of the diastereomeric salt pairs is minimal, which renders the agent non-selective according to the criteria that a difference of 3 kcal/mol or greater is required for agent selectivity. It can be hypothesized that the tail is an extremely flexible entity, which possesses the ability to twist and wrap itself around the binding pocket, excluding the space usually available for the ibuprofen molecule. Therefore, there is not a real difference in selectivity between the structures

1 and 2 in Table 7b as compared to the two same molecules, structures 2 and 3 in Table 7a, which possess the fluoroalkyl CO_2 philic tail.

Interestingly, structure 3 in Table 7b showed relatively high selectivity as compared to its fluoroalkyl modified counterpart. These results are suspect due to the inability of the force field employed to run a completed dynamics calculation. The values reported for that particular compound were determined from only the mechanics calculations. The value and the corresponding structure obtained from that calculation represent most likely a local minimum and not the lowest energy structure available for that molecule.

4.4.3 Effect of Lancaster Perfluoropolyether Based Tail on the Selectivity of L-Lysine

Because functional oligomers of hexafluoropropylene oxide at short chain lengths (3-5 repeat units) from Lancaster Chemical Co. can be obtained, such structures were included in the modeling study. The results for L-lysine modified with the 3 repeat unit perfluoroether tails are shown in Table 7c. The short perfluoroether tail, or more specifically perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl, as modeled in these calculations, possesses a molecular weight of 664 g/mol. This particular material is a recent addition to the perfluoroether family of compounds, which has been shown in our laboratories to impart good CO_2 solubility. [67]

As shown in Table 7c, the selectivity of these modified agents as compared to the native agent are all predicted to be significantly reduced, regardless of the placement of the perfluoroether tail. The same trends as those observed in the previous two cases are followed. Tail modification at the α amine results in higher predicted selectivity as compared to tail

placement at the ε amine. Placement of the tail so as to maintain both the α amine and the ε amine free for ion pairing exhibits a higher predicted selectivity at the α amine binding site than at the ε amine binding site. According to the selectivity criteria set forth in the previous section, none of the agents in Table 7c qualify for use in the resolution of racemic ibuprofen.

4.4.4 Effect of Tail Structure and Length on the Predicted Selectivity of the L-Lysine Derivatives

Intially, the fluoroalkyl tails seem to produce agents which would impart better selectivity than those possessing the perfluoro polypropylene oxide tails. The rigidity of the fluoroalkyl chain is greater than that of the perfluoro polypropylene oxide. This characteristic may assist in maintaining the proper, well defined, rigid environment necessary for chiral discrimination as opposed to a chain which exhibits greater mobility. The chain length in these calculations appears to have a strong influence over the selectivity of the agent, where as the tail length increases, the calculated selectivity decreases dramatically. From Tables 7a, 7b, and 7c, the only suitable agent determined theoretically for enantiomeric separation of ibuprofen is shown below in Figure 5.

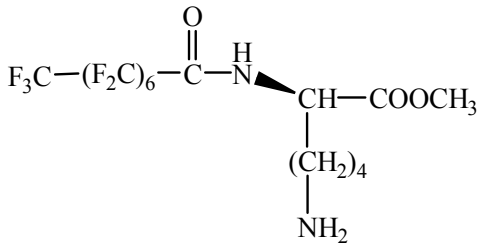


Figure 5 N-(Perfluorooctanoyl)-L-Lysine Methyl Ester

4.4.5 Effect of Perfluoroalkyl and Silicone Tails on Quinine Selectivity

Quinine has found extensive use as a resolving agent for racemic, chiral acids. [2] Ion pairing with quinine occurs at a tertiary amine located in the bicyclic portion of the molecule, as opposed to a primary amine in the case of L-lysine. This fact imparts a significant advantage in the use of quinine as a resolving agent for racemic ibuprofen over L-lysine in supercritical carbon dioxide. Primary amines, depending upon the temperature and pressure of the operating system and the pKa of the amine, can form carbamates with CO_2. This reaction is not observed when tertiary amines are utilized in high pressure carbon dioxide systems.

The computed selectivity for the native quinine molecule, determined as 3.0 kcal/mol, meets the minimum required value. Any chemical modification imparted to the native molecule will likely result in a reduction in the molecular selectivity as shown in Table 7d. Derivatization with a fluoroalkyl tail at the secondary alcohol group to create an ester functionality results in a 67% decrease in $\Delta\Delta E$. Here, the placement of the tail interferes with the binding of the ibuprofen molecule, not only through steric hindrance, but also by elimination of a primary hydrogen binding site at the asymmetric carbon.

In an effort to maintain the natural selectivity of the native molecule, another functional group located on the quinine molecule was sought for modification, and thus the vinyl group located far from the chiral center was considered. A silicone based tail was chosen, which can be easily accomplished synthetically via hydrosilation over a platinum catalyst. The computational results indicate reasonable preservation of the selectivity of the quinine molecule, most likely due to the preservation of an unrestricted binding pocket (structure 3 in Table 7d).

The last molecule under consideration, structure 4 in Table 7d, is the chiral inverse of quinine, namely quinidine. Interesting, this molecule does not exhibit the same degree of selectivity as quinine, possibly due to the geometrical configuration of the binding pocket. If the chiral secondary alcohol was not a vital component in the formation of the chiral complex, then each configuration, -(-) or –(+) (structures 1 and 4), would likely exhibit similar selectivity. This effect is not observed for structures 1 and 4. Thus, the secondary alcohol appears to be a necessary component in complex formation. This theory is reinforced by examining the fluoroalkyl derivatized agent of quinine, which displays a markedly reduced selectivity due to elimination of the hydrogen bonding potential of the secondary alcohol.

Of the potential agents for enantiomeric resolution from Table 7d, the silicone modified quinine agent exhibits the greatest potential for resolution of racemic ibuprofen. The structure of this molecule is shown below in Figure 6.

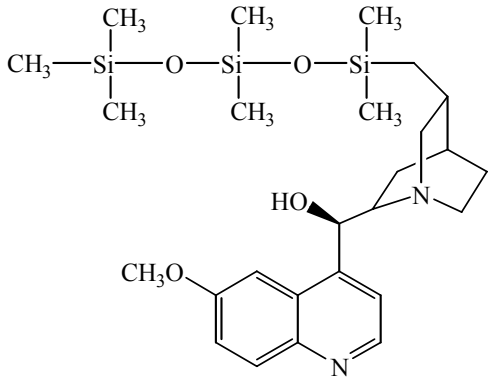


Figure 6 Silicone Functionalized Quinine

4.5 Concluding Remarks

These results highlight the first step towards the development of a custom designed, selective chiral resolving agent for use in the enantiomeric resolution of racemic ibuprofen in supercritical carbon dioxide. The force field employed, Allinger's MM2 has found sufficient use for the molecular geometries and energies of the complexes that were modeled in this study. This force field is well suited for small molecular systems which do not contain hybridization greater than sp^3 or metal ions. The major limitation in using Allinger's MM2 is the underestimation of hydrogen bonding by a factor of approximately three. Though the hydrogen bond is the primary bond between the components of the chiral complex, no substantial error results because it is the difference in steric energy rather than the absolute values which are of interest. This underestimation is assumed to be spread equally throughout the two diastereomeric salt pairs. Though these chiral complexes were modeled in vacuo, they give an indication of agents which are more likely to give good separation results even in supercritical carbon dioxide.

Table 7a L-Lysine Derivatized with Perfluoroalkyl Based Tail

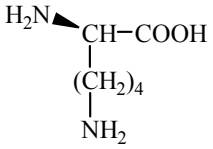
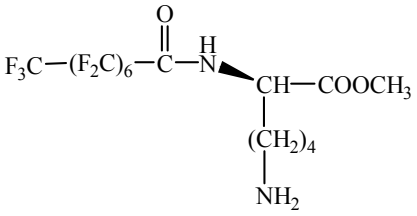
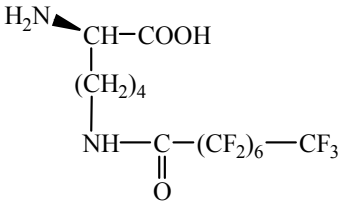
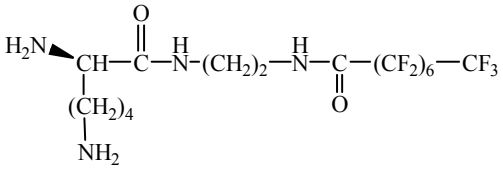
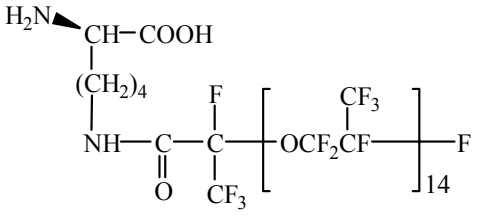
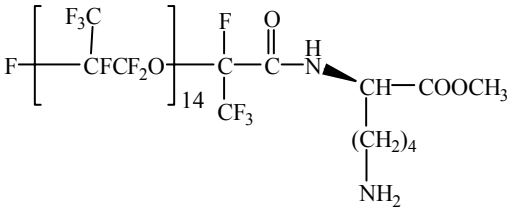
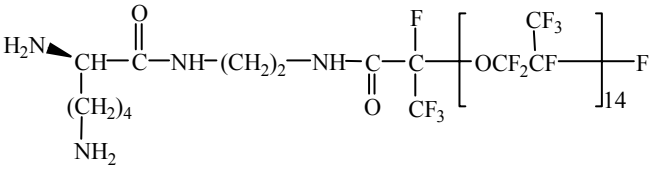
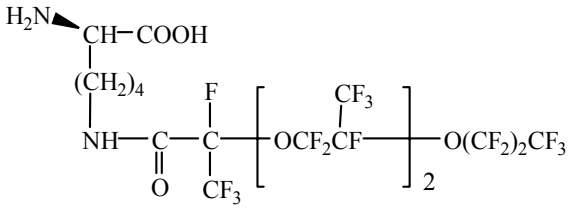
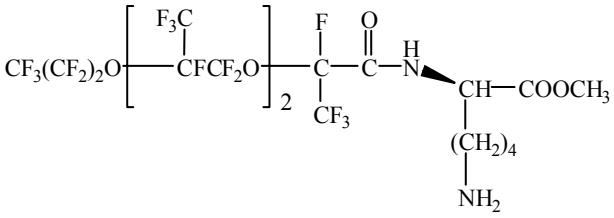
Resolving Agent	Δ Δ E (Kcal/mol) Ibuprofenate Complexes	Theoretical % Enantiomeric Excess
1. 	**4.86** * binding at e amine **0.61** * binding at a amine	**99.9** **64.4**
2. 	**3.82**	**99.8**
3. 	**0.56**	**61.2**
4. 	**0.62** * binding at e amine **2.35** * binding at a amine	**65.0** **98.1**

Table 7b L-Lysine Derivatized with Poly Hexafluoropropylene Oxide Based Tail

Resolving Agent	Δ Δ E (Kcal/mol) Ibuprofenate Complexes	Theoretical % Enantiomeric Excess
1. 	**1.86**	**95.7**
2. 	**1.22**	**87.3**
3. 	* NC * NC	**N/A** **N/A**

* NC = No Convergence

Table 7c L-Lysine Derivatized with Perfluoroether Based Tail

Resolving Agent	Δ Δ E (Kcal/mol) Ibuprofenate Complexes	Theoretical % Enantiomeric Excess
1. 	**0.69**	**68.9**
2. 	**0.44**	**52.5**
* This structure can be seen below the Table in the footnote.	**1.34** * binding at e amine **1.78** * binding at a amine	**89.6** **95.0**

* Structure was too large for cell area allocated.

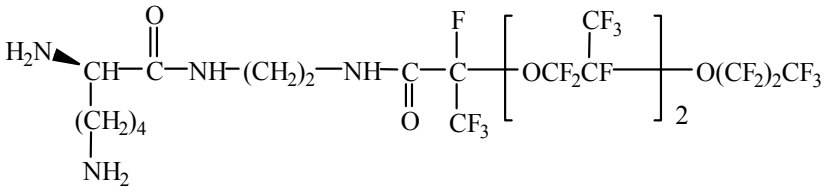
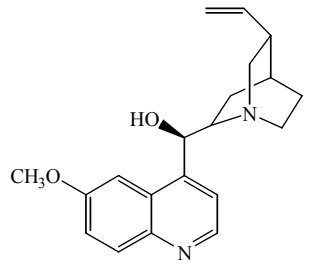
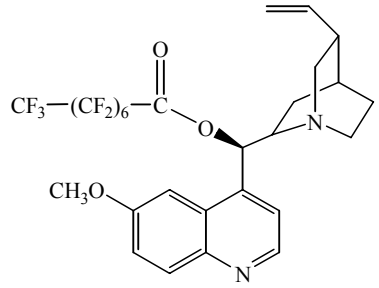
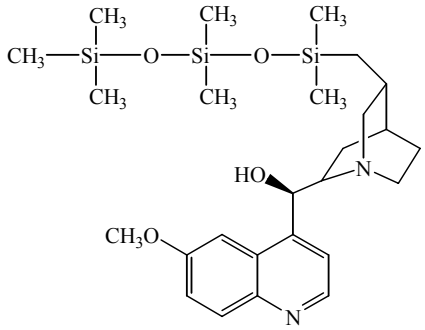
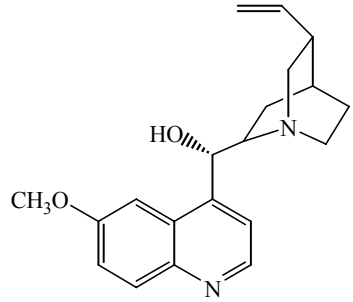


Table 7d Quinine Derivatized with Perfluoroalkyl and Silicone Based Tails

Resolving Agent	Δ Δ E (Kcal/mol) Ibuprofenate Complexes	Theoretical % Enantiomeric Excess
1. 	**3.0**	**99.3**
2. 	**1.07**	**81.2**
3. 	**2.65**	**98.8**
4. 	**1.22**	**87.3**

5.0 SYNTHETIC PROCEDURES FOR CUSTOM DESIGNED, CO_2 SOLUBLE RESOLVING AGENTS

Materials
The following is a list of reagents used as received, if not otherwise specified.

- Anhydrous tetrahydrofuran, anhydrous pentane, A.C.S. grade chloroform, A.C.S. grade n-hexane, A.S.C. grade methyl alcohol, 1,1,3-trifluorotrichloroethane, perfluoro 1,3-dimethylcyclohexane, A.C.S. grade dimethyl formamide, anhydrous toluene, triethylamine, pentadecafluorooctanoyl chloride, thionyl chloride, and palladium hydroxide 20 wt% Pd (dry basis) on carbon from Aldrich Chemical Company.

- Nε-CBZ-L-lysine methyl ester hydrochloride, Nα-CBZ-L-lysine, Nα,Nε-di-CBZ-L-lysine N-hydroxysuccinimide ester, quinine, and quinidine from Sigma Chemical Company

- Poly (hexafluoropropylene oxide) carboxylic acid terminated (MW = 2500) from Dupont

- Perfluoro 2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride from Lancaster Synthesis

- 1,1,1,3,3,5,5-heptamethyltrisiloxane and platinum divinyl tetramethyl disiloxane complex in xylene from Gelest
- Ethylene diamine from Fisher

- Nitrogen, argon, and hydrogen gases (high purity grade) from Liquid Carbonic

All products were characterized by ^1H NMR. Table 8 lists the structural characterization for each product.

Preparation of N-(Perfluorooctanoyl)-L-Lysine Methyl Ester

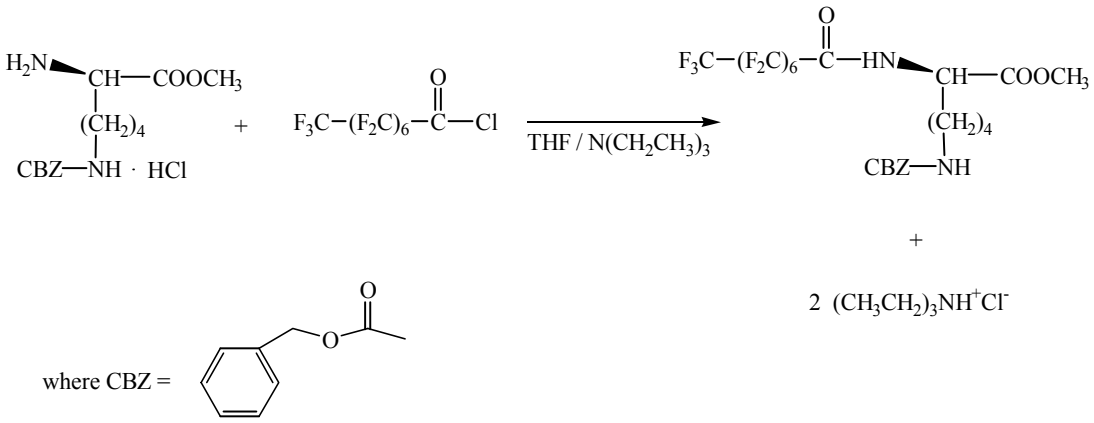


Figure 7 Reaction Scheme 1

Typically, 15 mmols (5.00 g) of Nε-CBZ-L-lysine methyl ester hydrochloride, 30 mmols (4.21 ml) of triethylamine, and 50 ml of anhydrous tetrahydrofuran were charged to a 250 ml, three neck flask which had been previously flushed with nitrogen. Subsequently, 15 mmols (3.75 ml) of pentadecafluorooctanoyl chloride in 10 ml of anhydrous tetrahydrofuran, was added dropwise with vigorous stirring over a 10 minute time period under nitrogen. After 10 minutes, the mixture was vacuum filtered and the filtrate then concentrated under vacuum. The white solid was then recrystallized from a 1:1 tetrahydrofuran/pentane solution. (Yield = 91.8%).

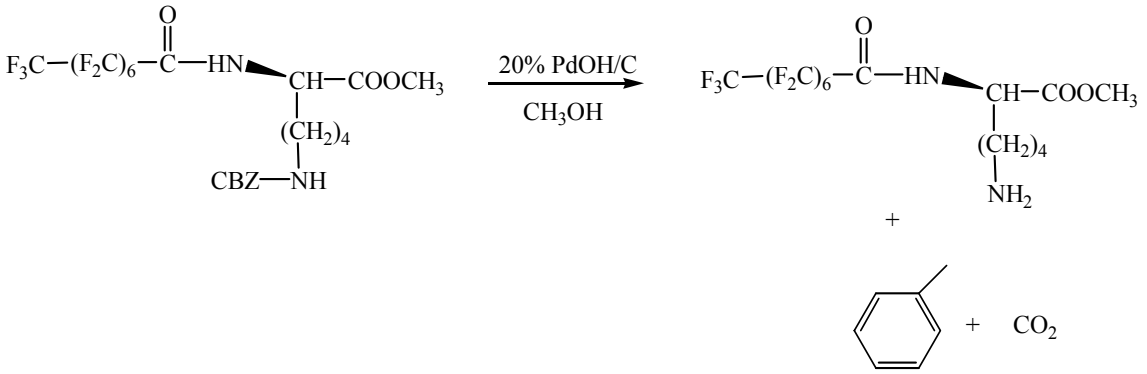


Figure 8 Reaction Scheme 2

To cleave the CBZ protecting group, a one arm flask was charged with 5 wt% of the palladium hydroxide on carbon catalyst in 5 ml of methyl alcohol. The flask was then evacuated and charged with hydrogen at 1 atm, and the mixture of 20 % palladium hydroxide on carbon catalyst, hydrogen, and methyl alcohol stirred for 10 minutes. The product from Reaction Scheme 1, 3.98 mmols (2.20 g), is dissolved in 10 ml of methyl alcohol and added to the flask. The flask is again evacuated, and the mixture is placed under a hydrogen blanket and stirred. After 4 hours, the catalyst is separated from the solution by filtration and the methyl alcohol removed under vacuum. The white solid was redissolved in tetrahydrofuran and recrystallized from a 1:1 mixture of tetrahydrofuran/pentane. (Yield = 53.0 %).

Preparation of N'- (Perfluorooctanoyl)-L-Lysine

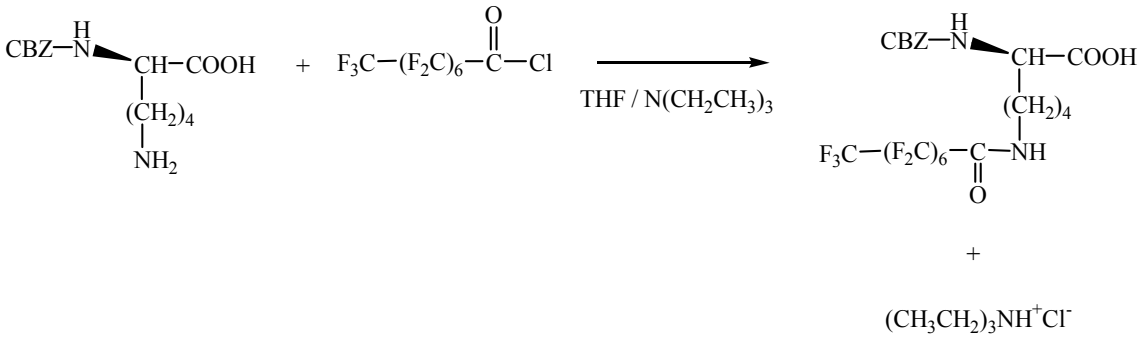


Figure 9 Reaction Scheme 3

The same synthetic procedure was followed as in Reaction Scheme 1. Typically, 10.7 mmols (3.00 g) of Nα-CBZ-L-lysine, 10.7 mmols (1.49 ml) of triethylamine, and 40 ml of anhydrous tetrahydrofuran were charged to a 250 ml, three neck flask. Subsequently, 10.7 mmols (2.65 ml) of pentadecafluorooctanoyl chloride in 10 ml of anhydrous tetrahydrofuran was added dropwise. The red solid was recrystallized from 1:1 chloroform/n-hexane solution. (Yield = 16.6 %)

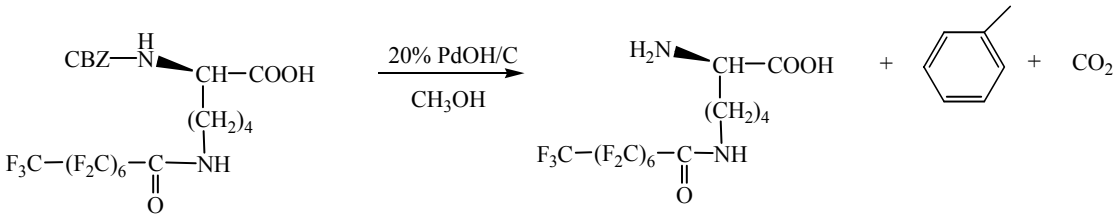


Figure 10 Reaction Scheme 4

The same synthetic procedure for CBZ deprotection was followed as in Reaction Scheme 2. Typically, the product from Reaction Scheme 3, 1.77 mmols (1.2 g), 0.065 g 20 % PdOH on carbon, and 15ml of methyl alcohol were added to a one arm flask. The product was a light red solid. (Yield = 35.2%)

Preparation of Perfluorooctanoyl L-Lysine with Alkyl Spacer

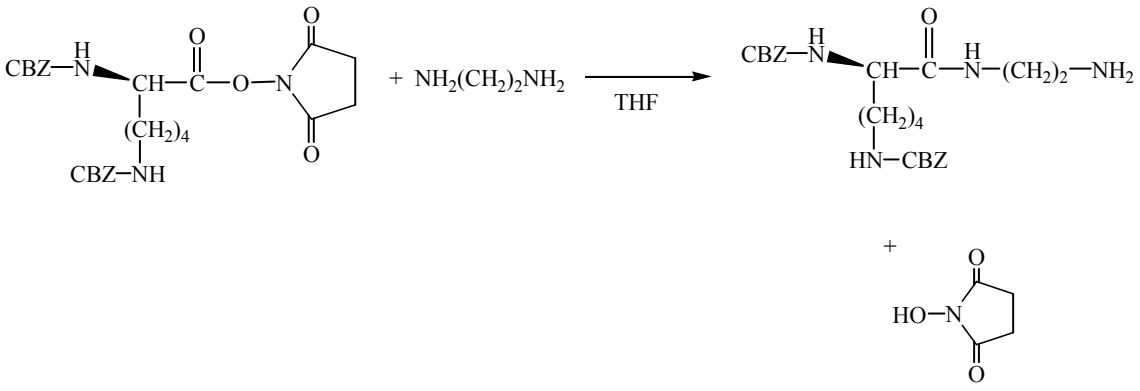


Figure 11 Reaction Scheme 5

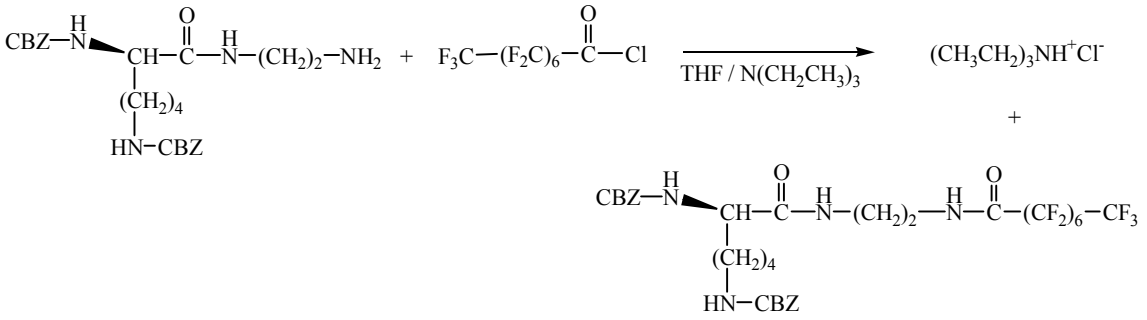


Figure 12 Reaction Scheme 6

Typically, 4.1 mmols (0.27 ml) of ethylenediamine in 10 ml of anhydrous tetrahydrofuran were charged to a 250 ml, three neck flask which had been previously flushed with argon. Subsequently, 4.1 mmols (2.09 g) of Nα,Nε-di-CBZ-L-lysine-N-hydroxysuccinimde ester in 50 ml of anhydrous tetrahydrofuran was added dropwise with vigorous stirring. After 20 minutes, a white precipitate formed. The reaction mixture was further diluted with 50 ml of anhydrous tetrahydrofuran and stirred vigourously. Then 3 mmols (0.74 ml) of pentadecafluorooctanoyl chloride in 10 ml of anhydrous tetrahydrofuran was added dropwise with vigorous stirring under argon. After 10 minutes, the mixture was vacuum filtered and the filtrate then concentrated. The white solid was then recrystallized from a 1:1 tetrahydrofuran/pentane solution. (Yield = 56.7%).

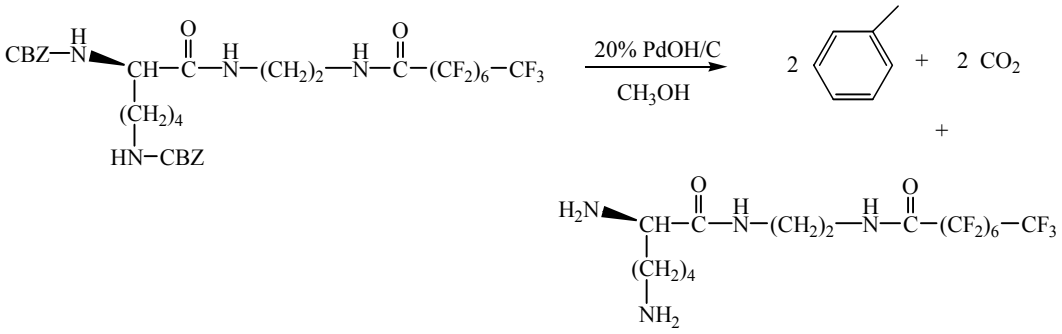


Figure 13 Reaction Scheme 7

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2. Typically, the product from Reaction Scheme 6, 2.29 mmols (2.0 g), 0.105 g 20% PdOH on carbon, and 15 ml of methyl alcohol were added to a one arm flask. The product was a yellow solid. (Yield = 39.2 %)

Preparation of N-(Polyperfluoroether)-L-Lysine Methyl Ester

Poly Hexafluoropropylene Oxide

To improve the "CO_2 philicity" of the functionalized lysine, analogues were prepared using an oligomer of hexafluoropropylene oxide (Trade name is Krytox, MW = 2500) capped with a carboxylic acid group. In order to increase its reactivity, the carboxylic group is converted to an acid chloride. In a typical experiment, 60 mmols (150 g) of poly (perfluoropropylene oxide), 50 ml of perfluoro -1,3-dimethylcyclohexane, 120 mmols (8.7 ml) of thionyl chloride, and 60 mmols N,N-dimethyl formamide (4.66 ml) were charged to a three neck flask, which was previously flushed with nitrogen. The reaction was heated under a nitrogen blanket to 76 ^{o}C, and the mixture was refluxed for 6 hours with vigorous stirring. The phases were then separated and

the solvent removed under vacuum. Conversion from the carboxylic acid to the acid chloride was characterized by FT-IR (disappearance of the acid peak at 1776 cm^{-1} and appearance of the acid chloride peak at 1807 cm^{-1}).

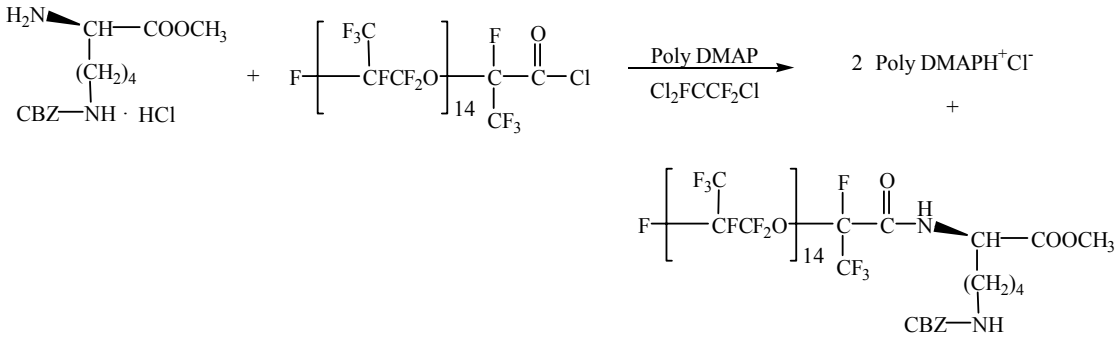


Figure 14 Reaction Scheme 8

Typically, 15 mmols (5.00 g) of Nε-CBZ-L-lysine methyl ester hydrochloride, approximately 2 g of Poly DMAP (crosslinked polymer of styrene and pryidine units), and 50 ml of 1,1,3-trichlorotrifluoroethane were charged to a 250 ml, three neck flask which had been previously flushed with nitrogen. Subsequently, 12.5 mmols (31.3 g) of poly hexafluoropropylene oxide (PHFPO) in 90 ml of 1,1,3-trichlorotrifluoroethane was added dropwise with vigorous stirring over a 2 hour time period under nitrogen. After 6 hours, the mixture is vacuum filtered and the filtrate then concentrated under vacuum. The product is washed with acetone, leaving a light yellow colored, viscous oil (Yield = 56.0 %).

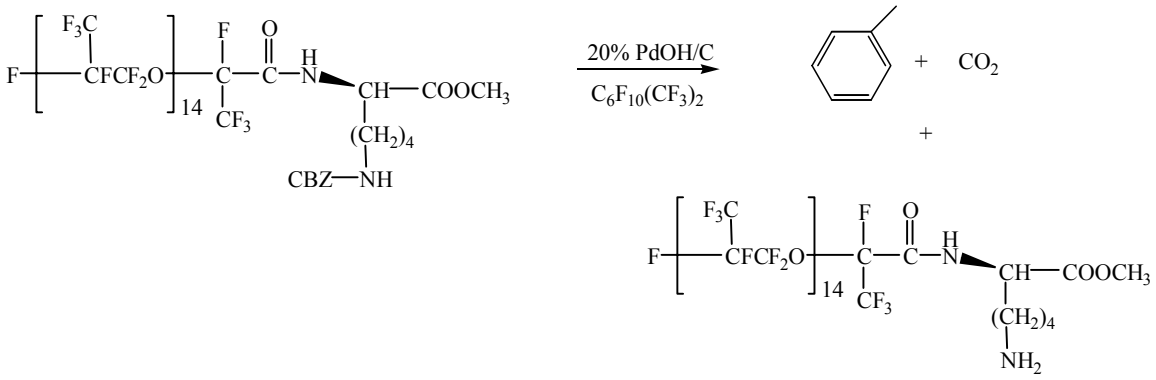


Figure 15 Reaction Scheme 9

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2 with a change in solvent. Typically, the product from Reaction Scheme 8, 1.20 mmols (3.2 g), 0.154 g 20% PdOH on carbon, and 15 ml of perfluoro 1,3 - dimethylcyclohexane were added to a one arm flask. The product was a clear, viscous oil. (Yield = 94.8 %).

Preparation of N'-(Polyperfluoroether)-L-Lysine

Poly Hexafluoropropylene Oxide

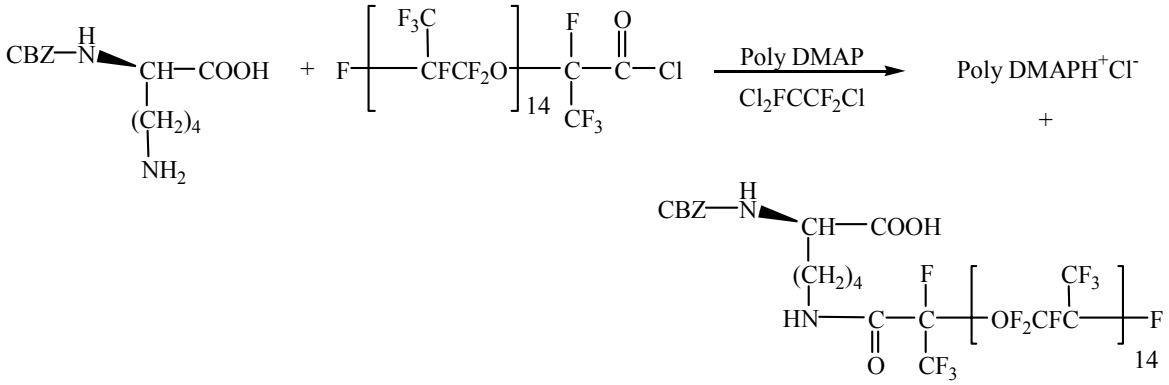


Figure 16 Reaction Scheme 10

The same reaction procedure was used as that described in Reaction Scheme 8. Typically, 17.8 mmols (5.00 g) of Nα-CBZ-L-Lysine, approximately 2 g of Poly DMAP, and 50 ml of 1,1,3-trichlorotrifluoroethane were charged to a 250 ml, three neck flask which had been previously flushed with nitrogen. Subsequently, 14.8 mmols (37.0 g) of PHFPO in 100 ml of 1,1,3-trichlorotrifluoroethane was added. The resultant product was a light yellow colored, viscous oil (Yield = 48.0 %).

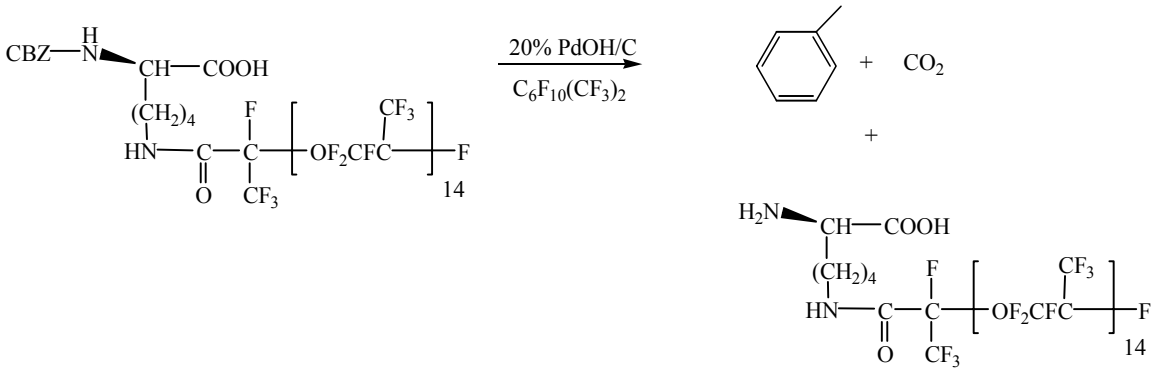


Figure 17 Reaction Scheme 11

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2 with a change in solvent. Typically, the product from Reaction Scheme 10, 1.28 mmols (3.4 g), 0.169g 20% PdOH on carbon, and 15 ml of perfluoro 1,3 - dimethylcyclohexane were added to a one arm flask. The product was a light brown, viscous oil. (Yield = 93.7 %).

Preparation of (Polyperfluoroether)-L-Lysine With Alkyl Spacer

Poly Hexafluoropropylene Oxide

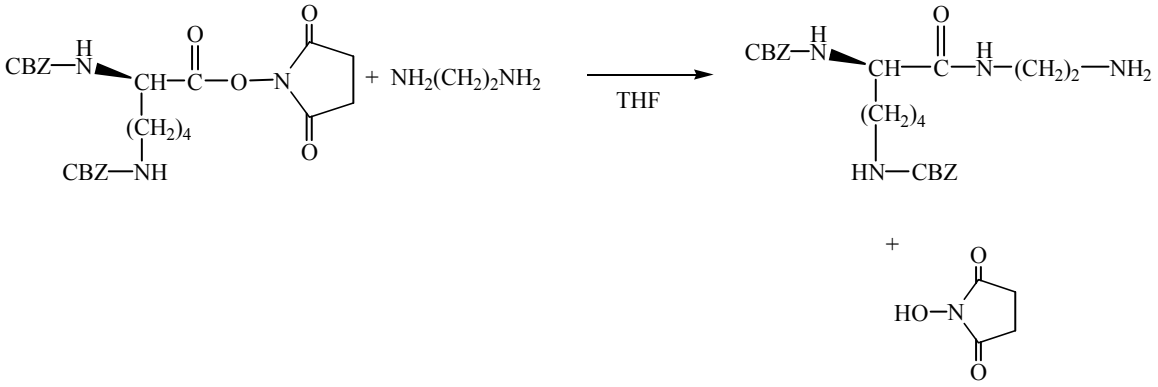


Figure 18 Reaction Scheme 12

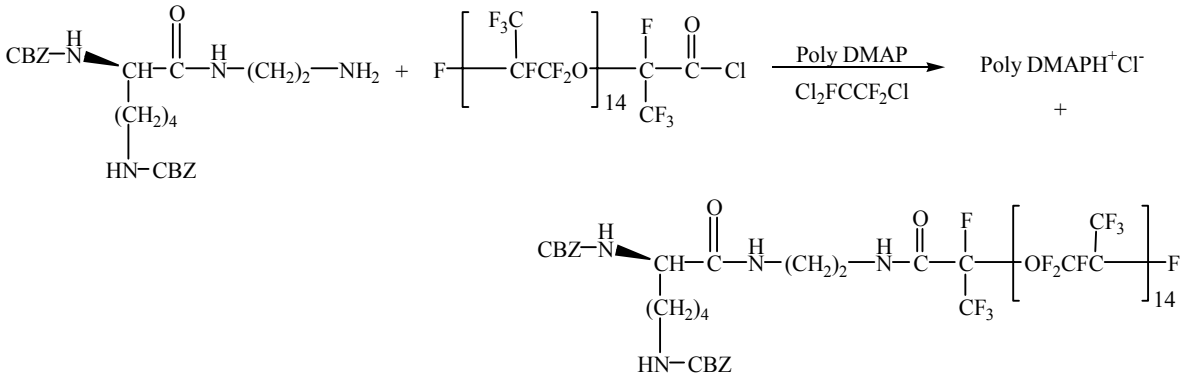


Figure 19 Reaction Scheme 13

Typically, 5.8 mmols (0.39 ml) ethylenediamine and 10 ml of anhydrous tetrahydrofuran were charged to a 250 ml, three neck flask which had been previously flushed with argon. Subsequently, 5.8 mmols (3.00 g) of Nα,Nε-di-CBZ-L-lysine-N-hydroxysuccinimde ester in 50

ml of anhydrous tetrahydrofuran was added dropwise with vigorous stirring. After 20 minutes, a white precipitate formed. The reaction mixture was further diluted with 100 ml of 1,1,3-trichlorotrifluoroethane and stirred vigorously. Then 2.9 mmols (7.25 ml) of PHFPO in 80 ml of 1,1,3-trichlorotrifluoroethane was added dropwise with vigorous stirring over a 2 hour time period under argon. After 6 hours, the mixture was vacuum filtered and the filtrate then concentrated. The product was washed thoroughly with acetone and resulted in a clear, pale yellow oil. (Yield = 62.0 %).

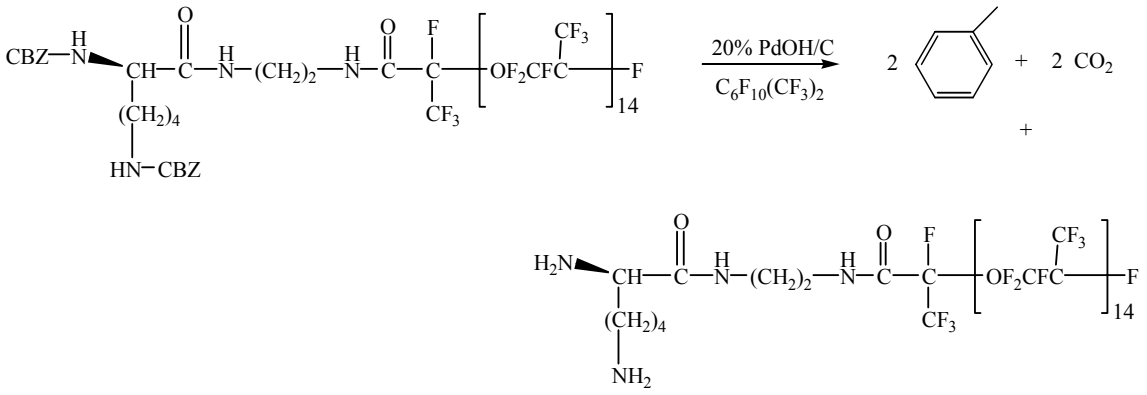


Figure 20 Reaction Scheme 14

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2 with a change in solvent. Typically, the product from Reaction Scheme 13, 1.9 mmols (5.3 g), 0.268 g 20% PdOH on carbon, and 20 ml of perfluoro 1,3 - dimethylcyclohexane were added to a one arm flask. The product was a yellow, viscous oil. (Yield = 82.1 %).

Preparation of N-(Polyperfluoroether)-L-Lysine Methyl Ester

Perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride

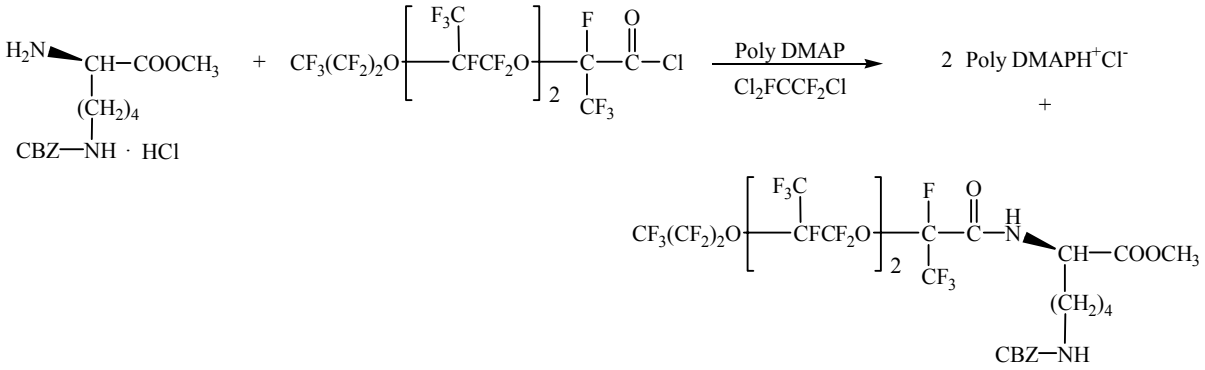


Figure 21 Reaction Scheme 15

A carboxy capped oligomer of hexafluoropropylene oxide available from Lancaster Synthesis, perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride (MW = 664), was also used as a tail to increase the "CO_2 philicity" of the lysine head group.

The same synthetic procedure is followed as in Reaction Scheme 8. Typically, 16.8 mmols (5.56 g) of Nε-CBZ-L-lysine methyl ester hydrochloride, approximately 2 g of Poly DMAP, and 60 ml of 1,1,3-trichlorotrifluoroethane were charged to a 250 ml, three neck flask which had been previously flushed with nitrogen. Subsequently, 14.0 mmols (9.30g) of perfluoro-2,5,8-trimethyl, 3,6,9-trioxadodecanoyl fluoride in 60 ml of 1,1,3-trichlorotrifluoroethane was added dropwise with vigorous stirring at 45 ^{o}C over a 1 hour time period under nitrogen. After 24

hours, the mixture is vacuum filtered and the filtrate then concentrated under vacuum. The resultant product was a light orange colored, viscous oil (Yield = 62.3 %).

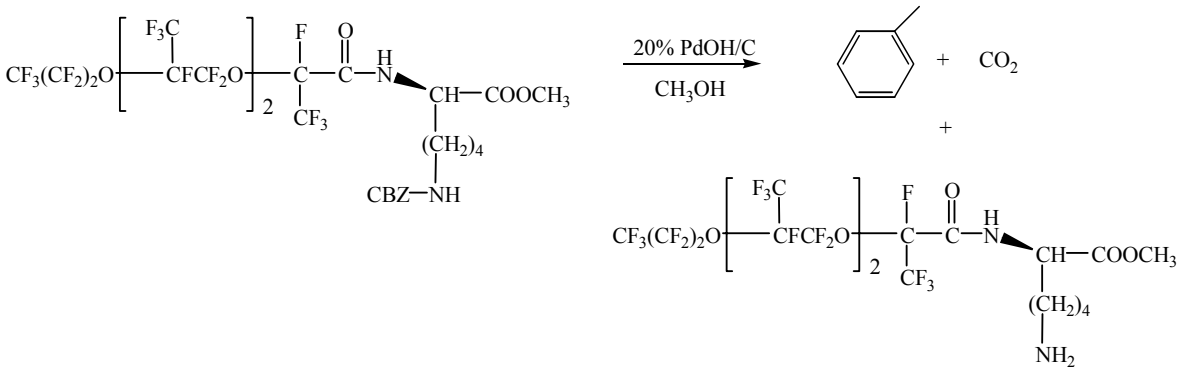


Figure 22 Reaction Scheme 16

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2. Typically, the product from Reaction Scheme 15, 2.6 mmols (2.5 g), 0.208 g 20% PdOH on carbon, and 20 ml of methyl alcohol were added to a one arm flask. The resultant product was a clear oil. (Yield = 85.4 %).

Preparation of N'-(Polyperfluoroether)-L-Lysine

Perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride

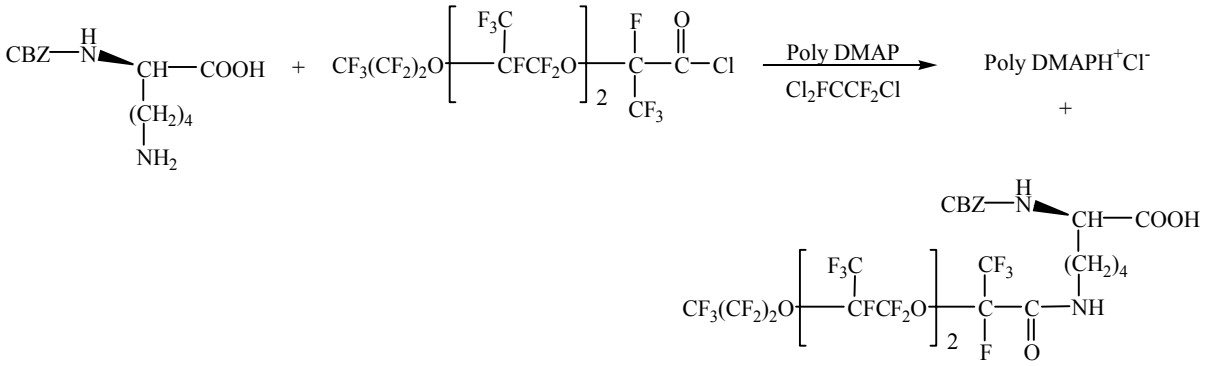


Figure 23 Reaction Scheme 17

The same reaction procedure was used as that described in Reaction Scheme 15. Typically, 17.9 mmoles (5.04 g) of Nα-CBZ-L-Lysine, approximately 2 g of Poly DMAP, and 60 ml of 1,1,3-trichlorotrifluoroethane were charged to a 250 ml, three neck flask which had been previously flushed with nitrogen. Subsequently, 14.6 mmols (9.72 g) of perfluoro-2,5,8-trimethyl, 3,6,9-trioxadodecanoyl fluoride in 60 ml of 1,1,3-trichlorotrifluoroethane was added. The resultant product was a light yellow colored oil. (Yield = 30.2 %).

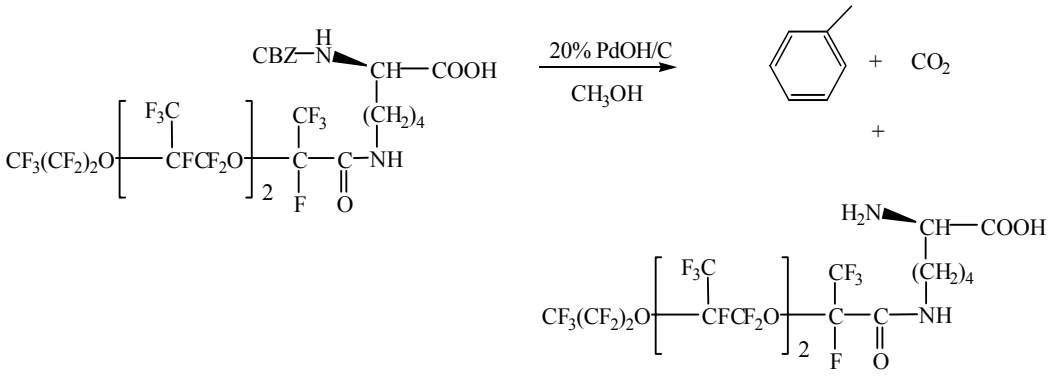


Figure 24 Reaction Scheme 18

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2. Typically, the product from Reaction Scheme 17, 2.4 mmols (2.3 g), 0.118 g 20% PdOH on carbon, and 20 ml of methyl alcohol were added to a one arm flask. The resultant product was a pale yellow oil. (Yield = 66.9 %).

Preparation of (Polyperfluoroether)-L-Lysine With Alkyl Spacer

Perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride

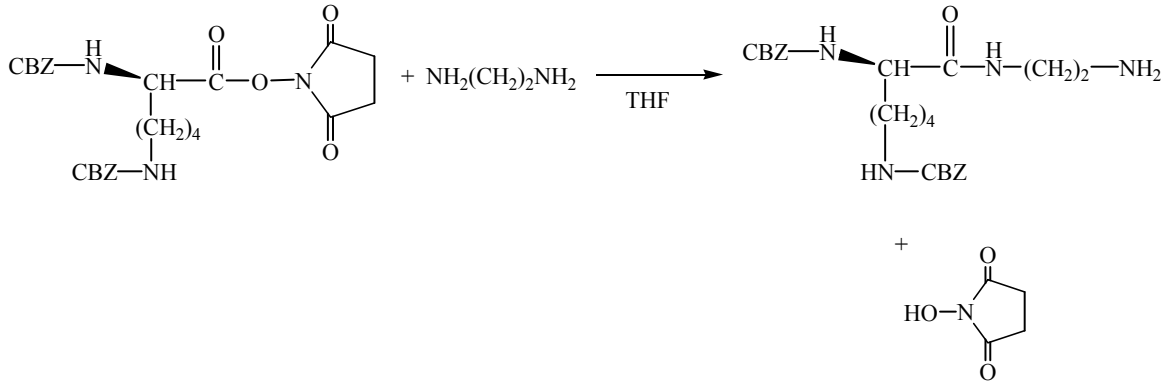


Figure 25 Reaction Scheme 19

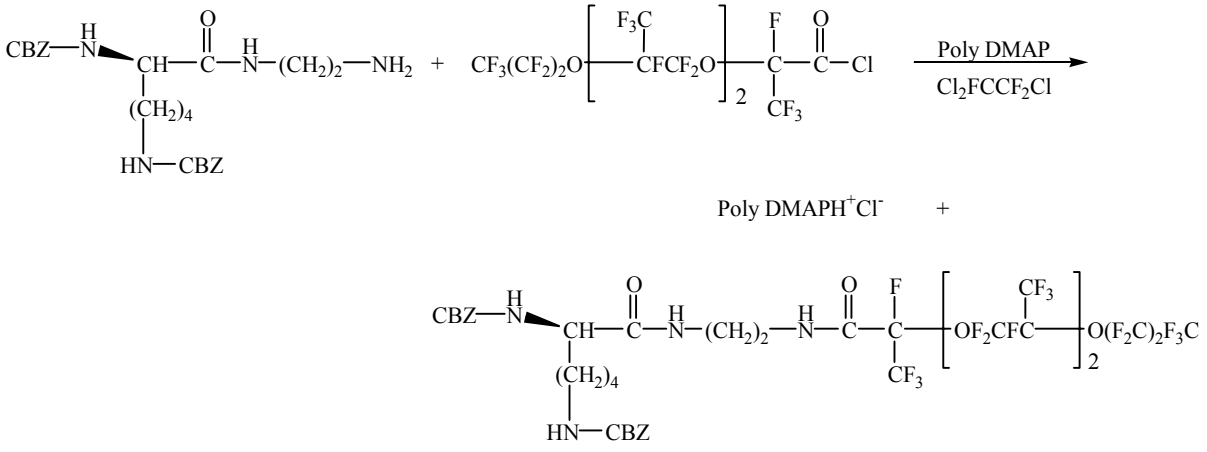


Figure 26 Reaction Scheme 20

The same synthetic procedure was used as described in Reaction Scheme 13. Typically, 3.9 mmols (0.26 ml) of ethylenediamine and 10 ml of anhydrous terahydrofuran were charged to a 250 ml, three neck flask which had been previously flushed with argon. Subsequently, 3.9 mmols (2.00 g) of Nα,Nε-di-CBZ-L-lysine-N-hydroxysuccinimde ester in 50 ml of anhydrous tetrahydrofuran was added dropwise with vigorous stirring. After 20 minutes, a white precipitate formed. The reaction mixture was further diluted with 100 ml of 1,1,3-trichlorotrifluoroethane and stirred vigorously. Then 3.0 mmols (1.99 g) of perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride in 50 ml of 1,1,3-trichlorotrifluoroethane was added dropwise with vigorous stirring over a 1 hour time period under argon. After 24 hours, the mixture was vacuum filtered and the filtrate then concentrated. The product was washed thoroughly with acetone and resulted in a clear, pale yellow oil. (Yield = 48.2 %).

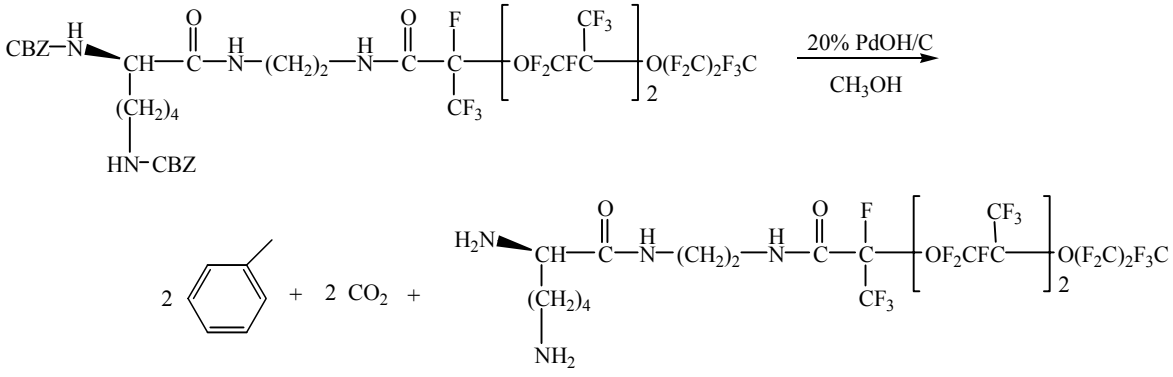


Figure 27 Reaction Scheme 21

The same reaction protocol was followed for CBZ deprotection of an amine as described in Reaction Scheme 2. Typically, the product from Reaction Scheme 20, 0.70 mmols (0.8 g), 0.062 g 20% PdOH on carbon, and 15 ml of methyl alcohol were added to a one arm flask. The resultant product was a yellow oil. (Yield = 75.5 %).

Preparation of Fluoroalkyl Functionalized Quinine

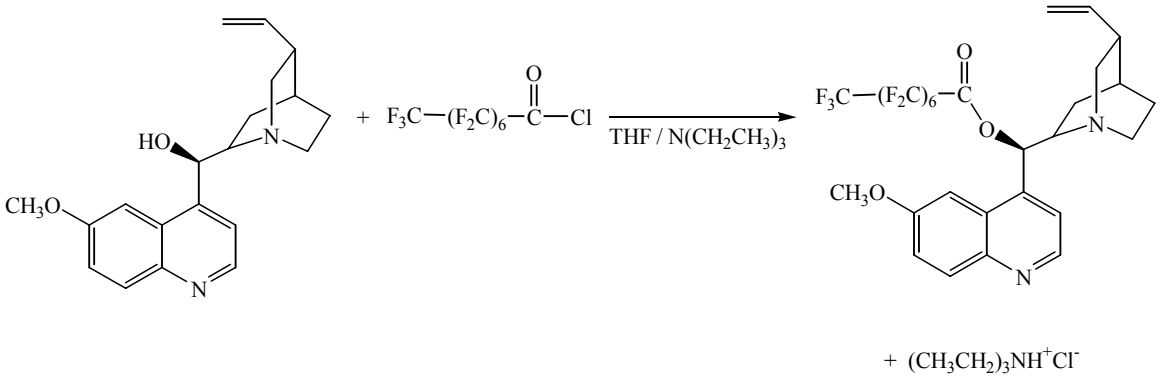


Figure 28 Reaction Scheme 22

In a typical experiment, 9.2 mmols (2.98 g) of quinine and 9.2 mmols (1.28 ml) of triethylamine in 30ml dry tetrahydrofuran were charged to a 3 neck, 250 ml flask which had been previous flushed with nitrogen for 15 minutes. Subsequently, 9.2 mmols (2.28 ml) pentadecaperfluorooctanoyl chloride in 10 ml dry tetrahydrofuran was added dropwise with vigorous stirring for 30 minutes at room temperature. The contents of the reaction mixture were

filtered under vacuum and the filtrate collected and concentrated. The resultant white, solid product was recrystallized from a 1:1 tetrahydrofuran/pentane solution. (Yield = 75.0 %).

Preparation of Fluoroalkyl Functionalized Quinidine

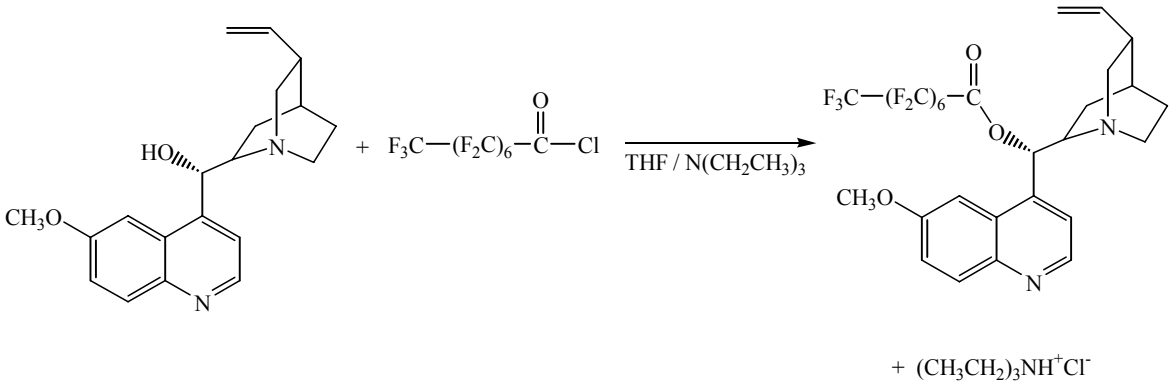


Figure 29 Reaction Scheme 23

The purpose of preparing this compound was to determine if this material showed preferential binding to the opposite enantiomer than quinine. The same synthetic procedure was used for the preparation of this reagent as in the preparation of the fluoroalkyl functionalized quinine. In a typical experiment, 6.4 mmols (2.07 g) of quinidine, 6.4 mmols (0.89 ml) triethylamine, and 6.4 mmols (1.59 ml) of pentadecaperfluorooctanoyl chloride were used. The product obtained was a white solid. (Yield = 36%).

Preparation of a Silicone Functionalized Quinine

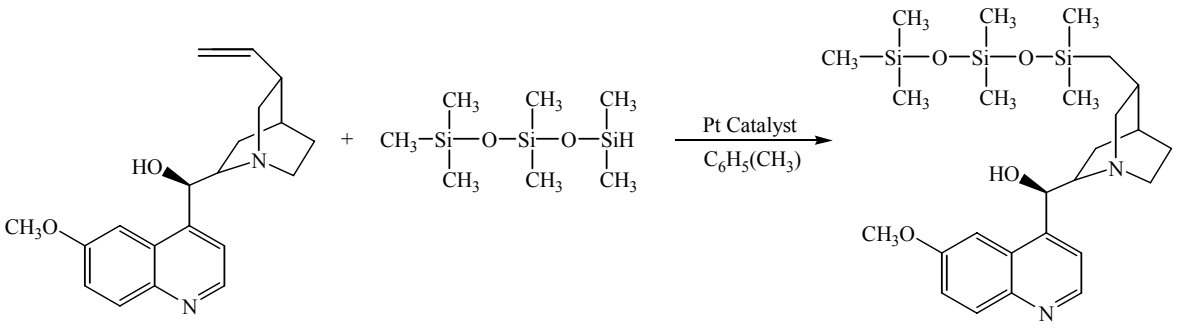


Figure 30 Reaction Scheme 24

In a typical experiment, a 3 neck, 250 ml flask is heat dried and flushed with nitrogen for 15 minutes. To the flask is added 15.4 mmols (4.99 g) quinine, 18.5 mmols (4.11 g) 1,1,1,3,3,5,5-heptamethyltrisiloxane, 0.0155 mmols platinum catalyst, and 50 ml dry toluene. The reaction mixture is again flushed with nitrogen and placed under a nitrogen blanket at 65 ^{o}C. The reaction is vigorously stirred for 3 days with incremental amounts of catalyst added, then left to cool and concentrated under vacuum. The product was purified by column chromatography using silica as the stationary phase and toluene as the eluent. The resulting material was then stirred for 4 hours at 60 °C in toluene with activated carbon. A pale brown oil resulted (Yield = 37%).

Table 8 Structural Characterization by ^{1}H NMR

Reaction Scheme	Head Group	Tail	NMR (ppm)
1	Ne-CBZ-L-lysine methyl ester	Fluoroalkyl	1.2-1.9, 3.1, 4.6, 5.1, 6.4, 7.2, 8.8
2	L-lysine methyl ester	Fluoroalkyl	1.2-1.9, 3.1, 4.6, 6.4, 8.9
3	Na-CBZ-L-lysine	Fluoroalkyl	1.3 - 2.1, 4.1, 5.1, 6.9, 7.4, 9.5, 12.1
4	L-lysine	Fluoroalkyl	1.3 - 2.1, 4.2, 6.9, 12.1
5	Na,Ne-CBZ-L-Lysine w/ EDA spacer	none	1.2 - 2.2, 2.8, 3.2, 4.3, 5.0, 5.7, 6.1, 7.4, 8.0
6	Na,Ne-CBZ-L-Lysine w/ EDA spacer	Fluoroalkyl	1.2 - 2.2, 2.8, 4.3, 5.1, 5.7, 5.8, 6.1, 7.4, 8.0
7	L-Lysine w/ EDA spacer	Fluoroalkyl	1.2 - 2.2, 2.7, 4.2, 5.8, 6.1, 8.1
8	Ne-CBZ-L-lysine methyl ester	PHFPO	1.4-1.8, 3.6, 4.5, 5.0, 6.4, 7.2, 8.0
9	L-lysine methyl ester	PHFPO	1.4-1.9, 2.9, 3.7, 4.5, 8.0
10	Na-CBZ-L-lysine	PHFPO	1.4 - 2.0, 4.2, 5.0, 6.7, 7.2, 8.0, 12.7
11	L-lysine	PHFPO	1.5 - 2.1, 3.4, 4.0, 6.0, 13.6
12	Na,Ne-CBZ-L-Lysine w/ EDA spacer	none	1.2- 2.5, 2.9 -3.2, 3.4, 4.3, 5.1, 5.7, 6.9, 7.3, 8.0
13	Na,Ne-CBZ-L-Lysine w/ EDA spacer	PHFPO	1.2- 2.5, 2.9 -3.3, 4.3, 5.1, 5.7, 5.9, 6.9, 7.3, 8.0
14	L-Lysine w/ EDA spacer	PHFPO	1.2- 2.5, 2.9 -3.2, 3.4 - 3.8, 4.3, 5.7, 5.9

Table 8 (Continued).

Reaction Scheme	Head Group	Tail	NMR (ppm)
15	Ne-CBZ-L-lysine methyl ester	PTTF	0.9 - 1.8, 3.8, 4.5, 5.0, 6.1, 7.4, 7.9
16	L-lysine methyl ester	PTTF	0.9 - 1.8, 2.3, 3.8, 4.5, 7.9
17	Na-CBZ-L-lysine	PTTF	0.9 - 1.9, 4.2, 5.0, 6.6, 7.2, 12.1
18	L-lysine	PTTF	0.9 - 1.9, 2.4, 3.4, 4.2, 6.6, 12.0
19	Na,Ne-CBZ-L-Lysine w/ EDA spacer	none	0.9 - 2.2, 3.7, 4.3, 5.1, 5.9, 6.6, 7.3, 8.4
20	Na,Ne-CBZ-L-Lysine w/ EDA spacer	PTTF	0.9 - 2.2, 4.3, 5.1, 5.7, 5.9, 6.6, 7.3, 8.4
21	L-Lysine w/ EDA spacer	PTTF	0.9 - 2.2, 3.1 - 4.1, 4.3, 5.9, 6.6
22	Quinine	Fluoroalkyl	1.25 - 3.42, 3.9, 4.0, 4.3, 4.9, 5.7, 7.2-7.9, 8.6
23	Quinidine	Fluoroalkyl	1.25 - 3.42, 3.9, 4.0, 4.3, 4.9, 5.7, 7.2-7.9, 8.6
24	Quinine	Silicone	1.25 - 3.42, 3.9, 4.0, 4.3, 5.7, 7.2-7.9, 8.6

Note: PHFPO = polyhexafluoropropylene oxide

PTTF = perfluoro-2,5,8-trimethyl-3,6,9-trioxadodecanoyl fluoride

6.0 EXPERIMENTAL HIGH PRESSURE AND PHASE BEHAVIOR ANALYSIS EQUIPMENT

6.1 W.B. Robinson Cell

The W.B. Robinson Cell is a commercial apparatus which is used for the static measurement of a material's phase behavior in supercritical fluids. Unlike a dynamic system, a static setup is usually used to determine phase border curves in P-T space and solubility of non-volatile compounds. In all static systems the primary components are a high pressure, variable volume view cell and some form of an optic system. A general schematic of the W.B. Robinson Cell is shown in Figure 31. The advantages of using this static system for cloud point evaluation are;

1) phase transitions are easily determined by visual inspection
2) sampling is not required
3) small sample size
4) continual adjustment of the pressure at a fixed temperature
5) sample recovery

6.2 High Pressure Batch Reactor

A schematic of the high pressure, custom designed batch reactor used in this project is shown in Figure 32. This reactor features sapphire view windows (General Ruby and Sapphire

Co.) for visual observation of phase splitting, a high pressure trap (Thar Designs) for the collection of precipitated materials, a high pressure sampling valve with a 500 μl sample loop (Valco Instruments), and heating rods (Omega Engineering) contained within the body of the reactor. This reactor configuration was used to experimentally measure high pressure equilibrium constants by assessing the fluid phase concentration of ibuprofen. The high pressure system shown in Figure 33 is a modification of the reactor system shown in Figure 32. The addition of the downstream depressurization/trapping system was incorporated in order to perform fractionation experiments. The major components incorporated into this system included a back pressure regulator (Tescom) and a high pressure trap (Thar Designs).

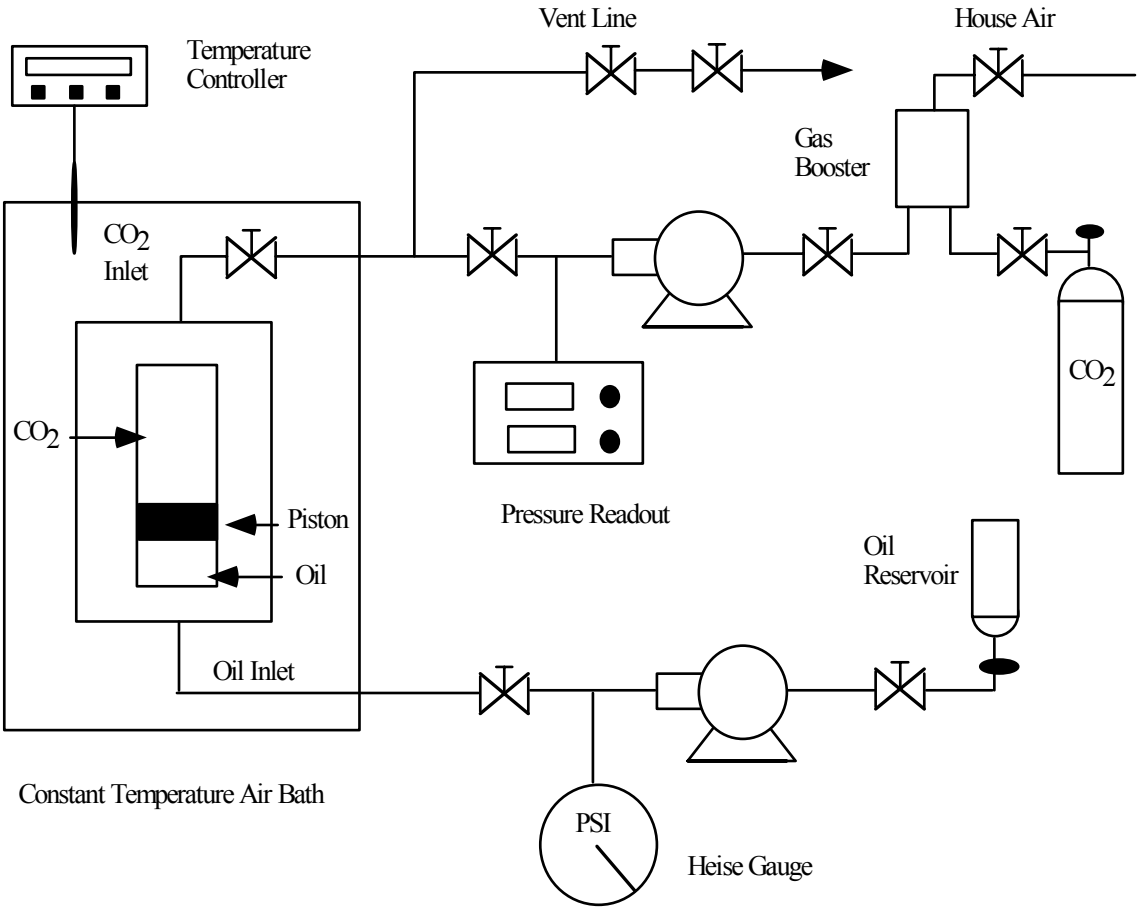


Figure 31 W.B. Robinson Variable Volume View Cell

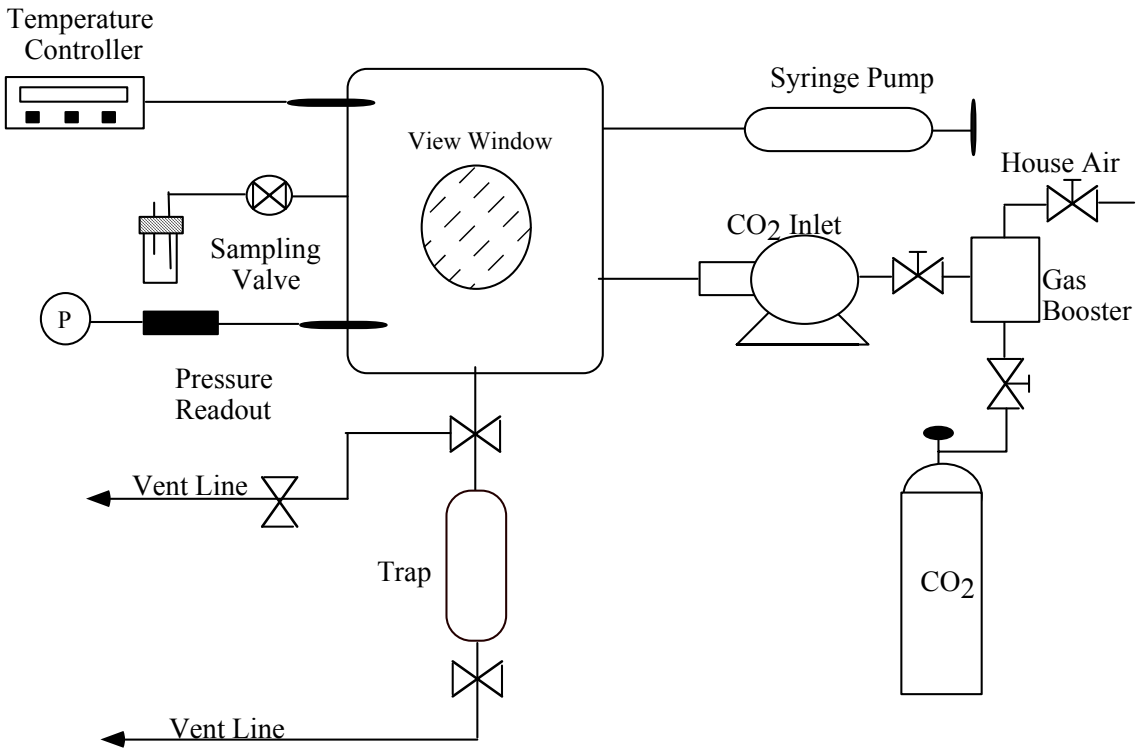


Figure 32 High Pressure Batch Reactor with Sampling System

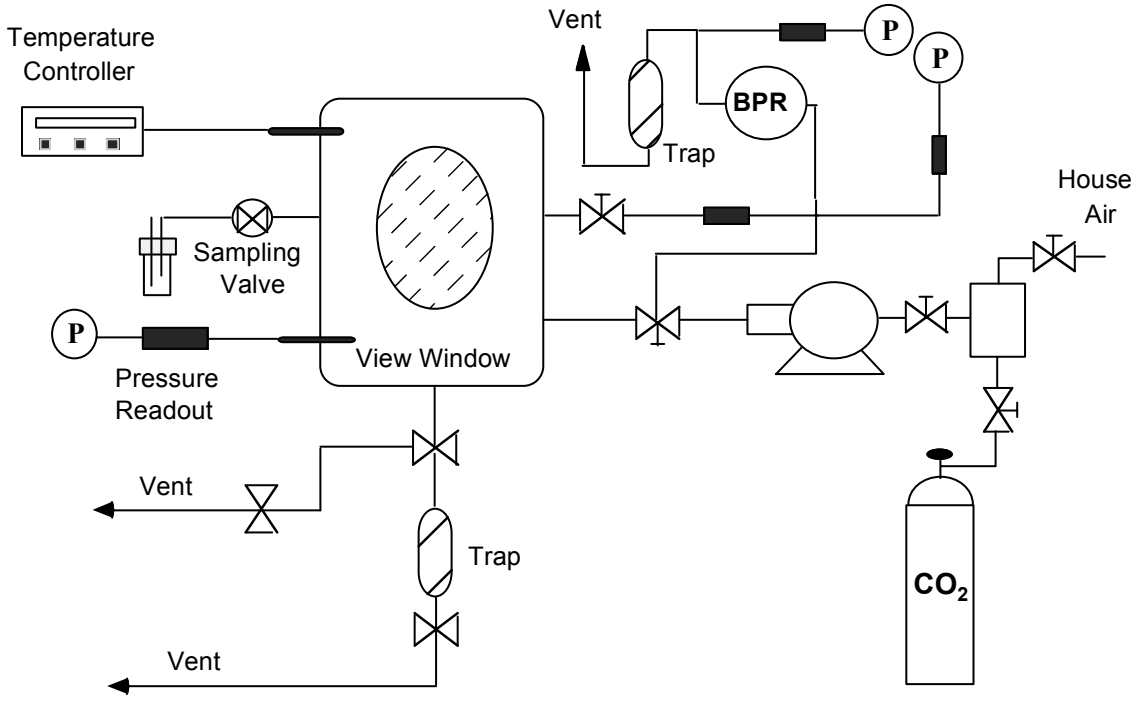


Figure 33 Modified High Pressure Batch Reactor

7.0 SOLUBILITY DETERMINATION IN SUPERCRITICAL CARBON DIOXIDE

7.1 Introduction

The solubility limits of the individual components in a supercritical fluid reaction system may be evaluated on the basis of their cloud points. In its thermodynamic definition, the cloud point is defined as the pressure/temperature point at which the chemical potentials of each component in the homogeneous phase become equal. In practice this translates for a supercritical fluid system to the pressure at which phase separation commences at a given temperature. [36]

The enantiomers of the free acid of ibuprofen exhibit nearly identical phase behavior in supercritical carbon dioxide. [68] In the form of their diastereomers, however, differences in phase behavior are anticipated due to energetic stabilization of the individual molecules and the ability of the solvent to solvate non-identical structures. Quantitative measurements of the phase behavior of these diastereomers provide not only valuable information regarding the individual solubility of these non volatile materials in supercritical CO_2, but also indicate the extent of difference in solubility between the two components. This solubility difference is what allows for a rational design for a selective extraction process.

There exists two methods by which to impart CO_2 solubility to insoluble materials. The first method is to add a miscible modifier, usually 2% to 10% v/v, to the fluid phase. The purpose is not to change the overall bulk properties of the fluid phase, but rather to aid in dissolving the analyte into the surrounding medium. Polar modifiers, such as methanol, form interactions like hydrogen bonding and induced dipole moments with the analyte to result in a

more favorable partitioning of the sample into the bulk supercritical fluid phase. [40] There are two distinct disadvantages of using modifiers. First, a characteristically benign medium is compromised due to the introduction of an organic constituent. Next, processing parameters are also altered. Product recovery not only will involve depressurization to remove the CO_2 gas, but also clean up of residual modifier present in the product, which increases processing costs and time.

To circumvent the use of organic solvents, chemical derivatization of the agent may be employed in order to attain solubility in CO_2. In this research project, we have used this method for the purpose of maintaining a benign reaction/separation medium. Two important chemical criteria must be applied to the native agents in order to render them suitable for enantiomeric resolutions. First, the derivitization process must be simple to perform and reactions conditions mild enough so as not to induce inversion of the chiral center, thus altering the agent's natural selectivity. The second criterion focuses on the structure of the natural agent itself. Though there does not exist a well-defined set of rules for enantioselectivity, empirical evidence suggests that functionalities close to the chiral center are important in defining a rigid, steric environment for selectivity to be determined. Thus, a resolving agent must possess a functionalizable group far removed from the chiral center. From the list of potential candidates in Table 4, L-lysine and quinine were selected. Employing similar chemical strategies as previously reported in our laboratories, families of functionalized L-lysines and quinines were synthesized whose structures are shown in Figures 34, 38, 39, and 40. [62,63,64,65,66]

7.2 Experimental Procedure

The W.B. Robinson Cell, as shown in Figure 31, is utilized to measure cloud points, which represents the boundary of phase separation. The main feature of this apparatus is a high pressure, variable volume view cell, which allows for visual determination of phase separation. The cell is initially loaded with a predetermined sample amount and aluminum mixing beads. An oil supply is pumped into the bottom of the cell so as to displace the piston containing the sample to the top of the view cell and remove residual air. A known amount of carbon dioxide is then added to the cell and the system is allowed to equilibrate at a designated temperature.

The cloud point is measured in the following manner. The entire cell is pressurized until dissolution and then rocked via a mechanically rotating arm until thorough mixing is achieved. The pressure is then slowly decreased until precipitation of the sample occurs. This may be seen visually by observing the transition from a clear fluid phase to one that becomes opaque. [75,78] Three measurements are taken for each dilution, the measurements are average, and a standard deviation is calculated.

7.3 Results and Discussion

The phase behavior of the chemically modified resolving agents in carbon dioxide is predominantly influenced by their physical architecture, thermodynamic properties, and carbamate formation. The sum of these processes determines the overall solubility of an agent in CO_2 and its reactivity in this medium. The physical effects are based upon thermodynamic

phenomena and the average weight that each parameter contributes varies between each family of agents. A brief description of each parameter follows.

7.3.1 Resolving Agent Architecture

The chiral resolving agents described in Chapter 5 are all composed of a polar, CO_2 phobic head group and a CO_2-philic tail. The tail's function is to impart CO_2 solubility, while the mission of the head group is to form specific chemical interactions with a target molecule. The phase behavior of these modified structures will thus be dependent in part upon the individual contributions of each segment. In particular, the degree of solubility of these compounds will vary significantly according to the extent of "CO_2 philicity" exhibited by each molecule as a whole. The "CO_2 philicity" of these resolving agents may be adjusted as a function of the tail's physical structure and chemical composition. For example, we have found that fluoroether, fluoroalkyl, and silicone-based tails are effective in conveying solubility in CO_2. The use of fluorinated esters has also been shown to be efficient in solubilizing polar materials. [71] Each tail type possesses functional groups that collectively exhibit unique physical properties and chemical interactions to produce characteristic phase behaviors in a compressed carbon dioxide environment.

In addition, the solubility of the modified resolving agents may also be adjusted as a function of the placement of the CO_2-philic tail within the molecule. Hancu and Beckman have shown that fluoroether tails (2500 MW) attached in three different positions through amidic linkages on anthroquinone produced isomers that showed significantly varied phase behavior. [76] Krukonis also observed similar behavior when investigating hydroxybenzoic acids in CO_2. [36]

Using these examples, it can be seen that the effect of resolving agent phase behavior is strongly dependent upon its architecture. The challenge, then, in designing the architecture of these resolving agents is to produce molecules that exhibit high degrees of solubility without compromising the specific solute-solute interactions required for ion pair formation. The thermodynamic rationale for these physical and chemical effects is described below.

7.3.2 Free Energy of Mixing

Solubility for a compound is favorable when the free energy of mixing, as described in general format below in Equation (7-1), is negative.

$$\Delta G_M = \Delta H_M - T\Delta S_M \tag{7-1}$$

For ideal solutions that obey Raoult's law, solute and solvent molecules exhibit similar shape and size. For these solutions, the ideal heat of mixing is assumed negligible and the free energy of mixing is proportional to the entropy of mixing. For solutions in which there is a significant difference between solute and solvent molecular size and the presence of inter/intra molecular forces, non ideal solution behavior results. In this instance, both ΔH and ΔS deviate from ideal behavior.

7.3.2.1 Entropy of Mixing The entropy of mixing is dependent upon a number of factors, including specific interactions, differences in free volume, chain flexibility, and molecular weight. The weight factor which each parameter assumes ultimately influences its phase

behavior and the resultant solubility upon mixing will determine, in this instance, an agent's utility in a compressed carbon dioxide environment.

The entropy of mixing is a sum contribution of a combinatorial and residual function as described below in Equation (7-2).

$$\Delta S_M = \Delta S^C + \Delta S^R \tag{7-2}$$

According to the lattice theory of fluids as proposed by Flory and Huggins, the combinatorial entropy of mixing per volume of solution may be expressed as

$$\Delta S_M = k \ln \Omega \tag{7-3}$$

where k is the Boltzman's constant and Ω is the number of possible combinations in which a solute may be distributed among solvent molecules. [82,86,91] In the case of relatively identical solvent and solute molecules, the number of possible ways in which n_2 solute molecules may be distributed among n_1 solvent molecules within a lattice consisting of $n_o = n_1 + n_2$ cells is defined by Ω as

$$\Omega = n_o!/n_1!n_2! \tag{7-4}$$

Substituting (7-4) into (7-3) gives the familiar expression for the entropy of mixing

$$\Delta S = -k(n_1 \ln N_1 + n_2 \ln N_2) \tag{7-5}$$

where n_1 and n_2 refer to the number of molecules of solvent and solute, and N_1 and N_2 their respective mole fractions. [82,91]

This expression is applicable to molecules of similar size. Long chain molecules, though, have traditionally exhibited deviations from ideal behavior, and these deviations are attributed to variances in the entropy of mixing. [90] In order to describe the behavior of long chain molecules, the solute molecule is now considered to be composed of xn_2 molecules, in which x is descriptive

of the number of chain segments equivalent in size to the solvent molecule. In this manner, Ω is redefined as

$$\Omega=[n_o!/(n_o - xn_2)!n_2!][(z-1)/n_o]^{n_2(x-1)} \quad (7\text{-}6)$$

where z is the lattice coordination number and the expression for the entropy of mixing becomes

$$\Delta S_M = -k\{n_1 \ln\left(\frac{n_1}{(n_1 + xn_2)}\right) + n_2 \ln\left(\frac{n_2}{(n_1 + xn_2)}\right) - n_2(x-1)\ln[(z-1)/e]\} \quad (7\text{-}7)$$

Applying Stirling's approximation and subtracting the physical effect of polymer disorientation, equation (7-7) reduces to the following expression. [82,85,86,87,88,89,91]

$$\Delta S_M = -k\left(n_1 \ln\left(\frac{n_1}{(n_1 + xn_2)}\right) + n_2 \ln\left(\frac{xn_2}{(n_1 + xn_2)}\right)\right) \quad (7\text{-}8)$$

It is evident from equations (7-6), (7-7), and (7-8) that as a solute molecule becomes larger, the number of possible combinations in which to arrange the solute molecule in the solvent decreases as compared to a smaller sized solute. This observation is described as a function of the solute's molecular weight and determined from the relation

$$x = M / \rho V_1^o \quad (7\text{-}9)$$

where M is the molecular weight, ρ is the density of the solute at the solution temperature, and V_1^o is the molar volume of the solvent. [89] Thus, the larger the molecule, the smaller the overall entropic contribution to the free energy of mixing due to the reduced number of possible arrangements of solvent molecules and solute segments.

The combinatorial entropy of mixing represents only the external arrangement of molecules. The residual entropy of mixing, on the other hand, accounts for differences in free volume and specific interactions between like and unlike molecules. For example, the solubility of a molecule in carbon dioxide may be described as a function of the difference in free volume

exhibited between CO_2 and the solute. During the solution process, carbon dioxide must condense and concentrate around the solute, resulting in a reduction in the free volume of carbon dioxide. As more carbon dioxide molecules concentrate around the solute, the solute's rotational flexibility is decreased, thereby decreasing the positive contribution to the entropy of mixing. [77] So as the difference in free volume between carbon dioxide and the solute decreases, an entropic price is paid for a favorable enthalpic contribution to the free energy of mixing. In this instance, there would be a delicate balance between the favorable enthalpic effects versus the loss in entropy for solution to be feasible.

One assumption made in Flory's liquid lattice theory is that the solution configuration is random, in other words, the solute molecule is distributed within the solvent in a random fashion. If preferential interactions occur between solute and solvent molecules or between the solute molecules themselves, the assumption of randomness becomes less applicable depending upon the extent of these interactions. Because the solution would take on a more ordered configuration, the entropy of mixing residual term decreases, and the resultant entropy of mixing is less favorable. [80, 82]

Chain flexibility is a parameter that is influenced by solute intermolecular associations and is reflected by the glass transition temperature, which is utilized as a descriptive measure for highly flexible molecules. [79] A smaller characteristic glass transition temperature implies a lesser degree of solute intermolecular forces. Since solute intermolecular forces would reduce the number of possible conformations that a solute could assume, the resultant effect on the phase behavior of the solute in CO2 may be assumed to follow the general observation that phase behavior is directly proportional to the glass transition temperature. In other words, as the Tg of

a solute increases, the pressures required to solvate the solute increase, reflective of a less soluble material.

Chain flexibility is also related to the free volume of the solute and is descriptive of the number of allowable, unrestricted solute confirmations in the solvent. The greater the flexibility exhibited by the solute, the greater the disorientation of the molecule, hence, the greater the free volume of the solute. Thus, the free volume difference between solute and solvent decreases since the free volume of the solute becomes similar to that of CO_2.

7.3.2.2 Enthalpy of Mixing. The enthalpic contribution to the free energy of mixing involves the balance between solvent-solvent, solute-solute, and solvent-solute interactions. This balance of forces is important in defining the optimal operating pressure and temperature required to solubilize a given solute. The enthalpy of mixing may in general be defined by Equation (7-10) below.

$$\Delta H = \chi_1 kTN_1\nu_2 \tag{7-10}$$

The term χ_1 represents the interaction energy per solvent molecule divided by the quantity kT, and the product $\chi_1 kT$ defines the difference in energy of the solvent molecule contained within a solute molecule as opposed to the same solute molecule in its pure state. [80, 84] The greater the difference in the interaction energy, the larger the heat enthalpy of mixing, which leads to a more negative free energy of mixing. Those specific interactions that define this energetic parameter are described in greater detail below.

Solvent-Solvent Interactions

Carbon dioxide is a non polar fluid which exhibits very weak van der Waals forces, low dielectric constant, lacks a dipole moment, and does not self associate. [75] Therefore, $CO_2 – CO_2$ interactions are relatively weak, and the strength of the solvent -solvent interaction may be considered constant at a given temperature. Though these interactions are not relatively strong, they nonetheless are significant due to the poor solvating properties of the gas.

Increasing the system pressure at constant temperature is one method of overcoming the effect of $CO_2 – CO_2$ interactions. By increasing the pressure, the free volume difference between solute and solvent is decreased potentially resulting in enhanced solubility. [92] In addition, the number of non-specific interactions between solute and solvent may also increase, again resulting in a potential improvement in solubility. Raising the pressure at constant temperature, however, is an impractical approach due to the elevated operating costs incurred at higher operating pressures.

The only practical means to attempt to reduce these interactions is to raise the operating temperature at constant pressure. The increased molecular motion associated with an increase in temperature may inhibit $CO_2 – CO_2$ interactions from forming. Note however, that by increasing the temperature at constant pressure, the free volume difference between solute and solvent increases, resulting in an unfavorable entropic effect. [92] Hence, there is no real effective mean of reducing CO_2-CO_2 interactions without compromising other thermodynamic factors.

Solute-Solute Interactions

Physical forces, such as London dispersion and van der Waals, play a critical role in a solute's characteristic phase behavior, in particular their contribution to solute-solute interactions. To decrease the effect of these interactions, a molecular structure may be designed

to limit the degree of intermolecular attractions between solute molecules, namely its van der Waal forces. The cohesive energy density is a parameter which is reflective of the degree of van der Waal forces inherently contained within a liquid/polymer. Solution will occur when the intermolecular attractive forces of solute and solvent are similar, and this phenomena is reflected in the well know Hildebrand solubility parameter. [99] Carbon dioxide exhibits far weaker van der Waal forces than traditional organic solvents and thus, solutes which can achieve the same limited intermolecular forces will easily solubilize in CO_2. [75]

As shown by O'Neill and coworkers, the incorporation of fluorine containing functional groups enhances polymer solubility. [78] Also shown by Yee and Fulton, the fluoro analogue of 1-butanol exhibited higher solubility than 1-butanol. They rationalized that because fluorines are so highly electronegative, they exhibit repulsive forces, thereby decreasing solute intermolecular attractions. [95] Thus, molecules that possess fluorine containing functional groups, such as poly(perfluoroethers), CF_2, and CF_3 groups, exhibit a smaller degree of attractive van der Waal forces, reflective of a low cohesive energy density which promotes dissolution into compressed carbon dioxide.

Chemical forces also play a significant role in a solvent's ability to solvate a given solute. One of the largest contributions to solute-solute interactions, hence potential decreases in solubility, is hydrogen bonding. The model compounds investigated in this research project possess many locations for hydrogen bonding within their physical structures. Ironically, the molecular structure of these molecules must fulfill both the requirements of solubility and hydrogen bonding. On the one hand, limited solute-solute interactions are needed to bring the resolving agents readily into solution. In contrast, diastereomeric ion pairing is based on at least one hydrogen bond, therefore, functional groups which participate in hydrogen bond formation

must be present. Both enthalpic and entropic penalties are paid due to the formation of a more ordered solution configuration and subsequent decreases in solvent-solute interactions

Hydrogen bonding in supercritical CO_2 has been well documented. O'Shea and coworkers utilized UV-visible spectroscopy to examine hydrogen bonding in the tautomeric equilibrium of 4-(phenylazo)-1-naphthol. [93] Infrared spectroscopy was employed to investigate hydrogen bonding with pyrrole by Hyatt, and the degree of intermolecular H-bonding with certain alcohols and their fluoro-analogues were investigated by Yee and Fulton. [94,95,97] Sigman and coworkers spectroscopically observed H-bonding in the solvatochromic indicators, 4-nitroaniline and N,N-diethyl-4-nitroaniline. [96] Van Alsten and coworkers examined the role of hydrogen bonding on solubility when cosolvents, such as methanol, were added with polycyclic solutes. [36]

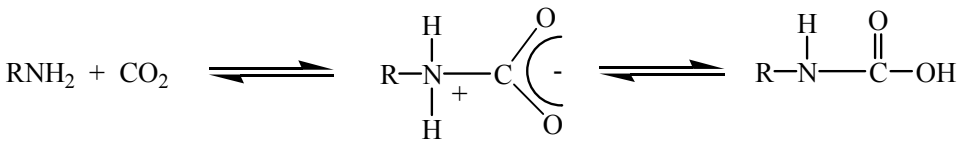
Hydrogen bonding has been recently investigated with polymer systems in CO_2. Kazarian and coworkers observed H-bonding in PVAc and Nylon 6. [71] Takishima and coworkers identified intramolecular and intermolecular hydrogen bonds, in which the oxygen of a hydroxyl functional group was bound to other hydroxy or ether oxygen atoms in PEG, PPG, and PEGDME polymers. [75] O'Neill and coworkers found that the primary alcohol groups in PEO polymers undergo hydrogen bonding, and the net effect was a dramatic increase in the operating pressure required to solvate the polymer. [78] Briscoe and Kelly observed hydrogen bonding occurring between an N-H and C=O functionalities in polyurethanes. [98] Thus, the common conclusion found in all of these studies, with the exception of those utilizing modifiers, is that the net effective solubility was penalized in systems that exhibited hydrogen bonding.

Solvent-Solute Interactions

An increase in the number of favorable carbon dioxide-solute interactions may be accomplished by incorporating functional groups, which exhibit a strong CO_2 interaction. Promoting solute-CO_2 interaction, via Lewis acid-base pairing, was shown by Kazarian and coworkers to be effective with electron donating groups such as carbonyl functional groups. [71,76,77] In a recent study by Sarbu and Beckman, a non fluorous CO_2-phile ether carbonate copolymer was synthesized and compared against a traditional poly(perfluoro) ether based polymer. Reported solubility measurements of these polymers indicated that the ether carbonate copolymer exhibited a higher degree of solubility than that of the poly(perfluoro) ether based polymer. These results give a strong indication of the preferential interaction of carbon dioxide for carbonyl functionalities. [79] A strong penalty, however, against the entropy of mixing is paid in terms of the difference in free volume. Though strong solvent-solute interactions contribute to a negative enthalpy of mixing and more favorable free energy of mixing, the effect is countered by a decrease in the entropy of mixing. By forming interactions, the randomness of the solution is decreased, thereby, rendering a less positive contribution to the entropy of mixing.

7.3.3 Carbamate Formation

Primary amines can react with carbon dioxide to form carbamate molecules as shown below in Equation (7-11).



(7-11)

Carbamates have traditionally served as nitrogen protecting groups in organic synthesis. [85] N-substituted carbamic acids are also relatively unstable molecules and can break down into carbon dioxide and its respective amine. [85] The formation of this complex is dependent upon many factors, which include CO_2 and amine concentration, pK_a of amine, temperature, and system pressure. Carbamate formation is also an equilibrium reaction in which the concentration of available amine is a function of temperature. For example, previous studies by Beckman and Diaf, who investigated the extent of carbamate formation occurring with free amines in styrene copolymers, have shown that in supercritical CO_2 the decomposition temperature of carbamate complexes occurs at approximately 55 ^{o}C. [69]

Empirical evidence suggests that the solubility of carbamate complexes formed from the resolving agents investigated in this project will be governed by the solubility of the CO_2 philic tail of the resolving agent. A largely CO_2 philic tail has the potential to solvate a carbamate complex since the carbamate portion of the molecule comprises only a small fraction of the molecule. Conversely, the carbamate complex will make a significant contribution to the solubility of the agent as a whole with agents that exhibit poorer solubility. Solubility of the agent, then, will be partially determined by the balance of the solvent interacting between the polar head group and the CO_2-philic tail of the agent molecule.

7.3.4 Quinine and Quinidine

Carbon Dioxide Philicity and Chiral Orientation

Quinine was modified with a fluoroalkyl and a silicone tail as shown in Figure 34, structures **1** and **2**. These tails exhibit low cohesive energy densities and are anticipated to impart favorable solubility to the native quinine based molecules. Although the silicone tail possesses a higher molecular weight than its fluoroakyl functional analog, the silicone derivatized agent exhibited cloud point pressures significantly below that of the fluoroalkyl. Here, although the use of a higher molecular weight silicone structure is entropically less favorable, the molecule is on balance more CO_2 philic and hence, dissolves at a lower pressure. One observation to note is that these tails are attached at different locations on the quinine molecule. Entropic effects pertaining to the ability of the agent to self associate and restrictions upon its rotational flexibility are expected to differ between the two agents. These effects, however, are difficult to independently discern given the high enthalpic favorability contributed by the silicone versus the fluoroalkyl tail.

Quinidine was derivatized in order to evaluate if the there is any effect on the solubility of a molecule as a function of its chirality. Racemic mixtures would not ordinarily be expected to exhibit differences in solubility in carbon dioxide, since it is a non chiral medium. For example, the phase behaviors of the enantiomers of ibuprofen were measured independently and no difference in phase behavior was evident. However, structure **3** in Figure 34 appears to show a significant difference in phase behavior from structure **2**, which is its chiral inverse. The

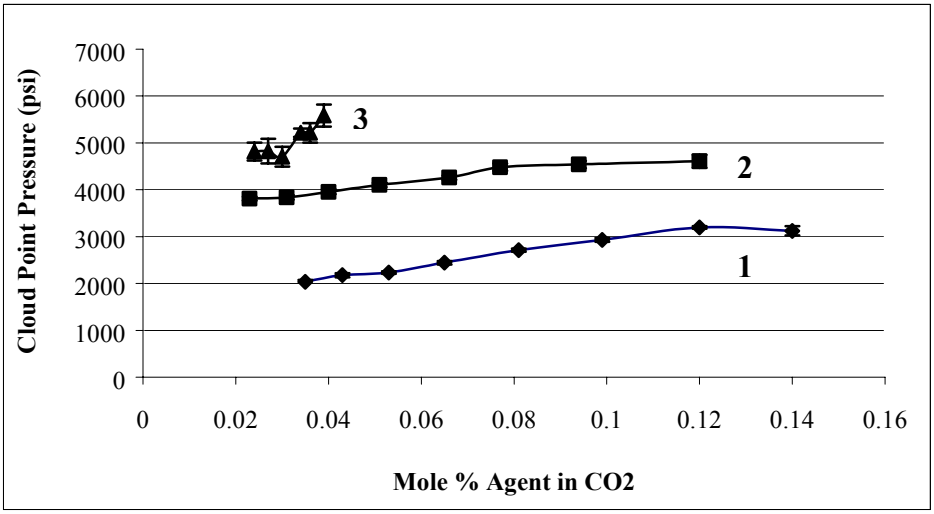
quinidine based resolving agent exhibits a marked decrease in solubility as compared to its quinine based analog.

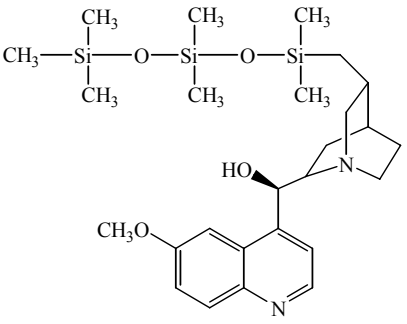
In this case, an argument may be made for differences in solute-solute interactions. Phenyl groups can undergo π–π bonding, producing structures which can orient themselves in a stacked configuration, thus altering the phase behavior in comparison to the same molecules randomly distributed in solution. Steric effects may also influence solute-solute interactions. As seen in molecules **2** and **3**, in close proximity to the phenyl group lays the fluoroalkyl tail. This tail may have the potential to interfere in the ability of a molecule to orient itself with respect to its neighboring molecule, depending upon its three dimensional alignment in space.

The preference of the quinidine analogue to self associate through hydrogen bonding is evident in its phase behavior. The absolute value of the free energy of mixing is negatively impacted from both an entropic and enthalpic viewpoint. Self association impacts the entropic contribution by causing a decrease in the rotational flexibility and subsequently the randomness of the solution. Higher solute-solute interactions as compared to the quinine analogue produces a more positive enthalpy of mixing. Hydrogen bonding has been noted to have a significant impact upon phase behavior as observed with isomers. [75, 77, 78] This leads to the conclusion that, though the quinine/quinidine agents are structurally similar, intermolecular interactions dominate the phase behavior and, thus, distinctly distinguishes the two molecules from one another in CO_2.

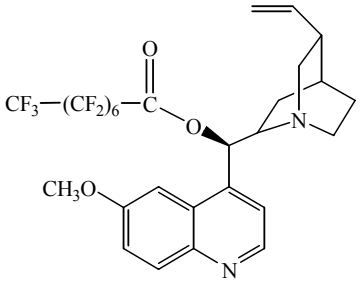
The error associated with each point on the cloud point curve was computed as a standard deviation. The standard deviations ranged from ± 30 psi to ± 262 psi. Based upon empirical evidence in our laboratories, computed standard deviations in the range of 50 to 300 psi are typical and the magnitude is operator dependent. Larger standard deviations were observed with the quinidine fluoroalkyl derivative. This is attributed to the cloud points observed near the

pressure limitations of the equipment. For this family of agents, it was also observed that the higher the solubility of the agent, the lower the calculated standard deviations. This trend was only observed among the quinine family. The range of standard deviations for agents of the various lysine families were relatively consistent, from approximately ± 10 to ± 130 psi.





(1)



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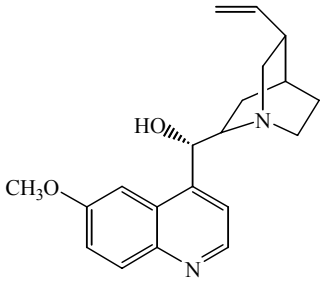


(3)

Figure 34 Quinine and Quinidine Fluoroalkyl and Silicone Derivatives

7.3.5 L-Lysine

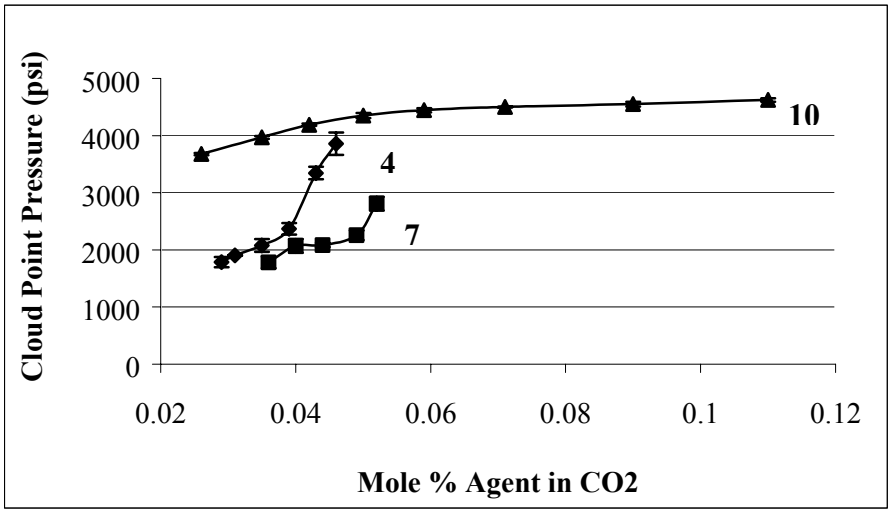
Comparison of Tail Length and Placement

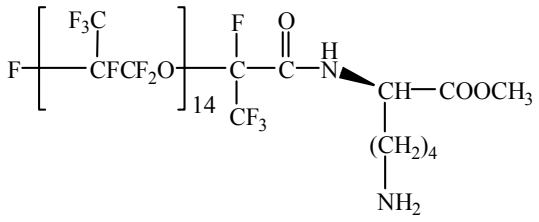
L-lysine possesses three potential sites for chemical derivitization located at the α and ε amines and the α carboxy functional group. Nine derivatives were generated in order to investigate any effect attributable to tail length and tail placement. Tail length was evaluated for one fluoroalkyl (MW = 432) and two perfluoroether tails differing in the number of repeat units (2 units versus 14 and a MW of 664 versus 2500 respectively).

Tail Length. Structures **4**, **7**, and **10** in Figure 35 is an interesting example of determining an optimal chain length for a given head group. By reason of molecular weight, structure **10** would be expected to result in the most soluble compound, followed by molecules **7** and **4**. What is observed, however, is that molecule **10** is actually the least soluble and **7** the most soluble. Molecule **10** possesses the fluoroalkyl tail, which is less "CO_2 philic" than the corresponding fluoroether chains, so its lower solubility in CO_2 is expected. Comparing molecules **4** and **7**, it is evident that entropic predominated over enthalpic effects. Though **4** possesses a greater gain in "CO_2 philicity", it is encumbered by its higher molecular weight as opposed to the lower molecular weight analog.

Examining molecules **5** and **8** in Figure 36, the same scenario was observed. Molecule **5**, which possesses a higher degree of "CO_2 philicity" is less soluble than molecule **8**. Again the

entropy of mixing predominates over solute $-CO_2$ interactions as a result of molecular weight differences. The same consistent result was observed for molecules **6** and **9** in Figure 37.





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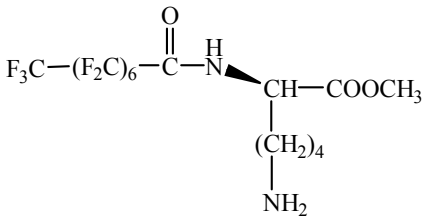

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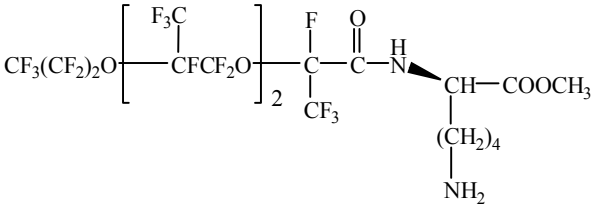

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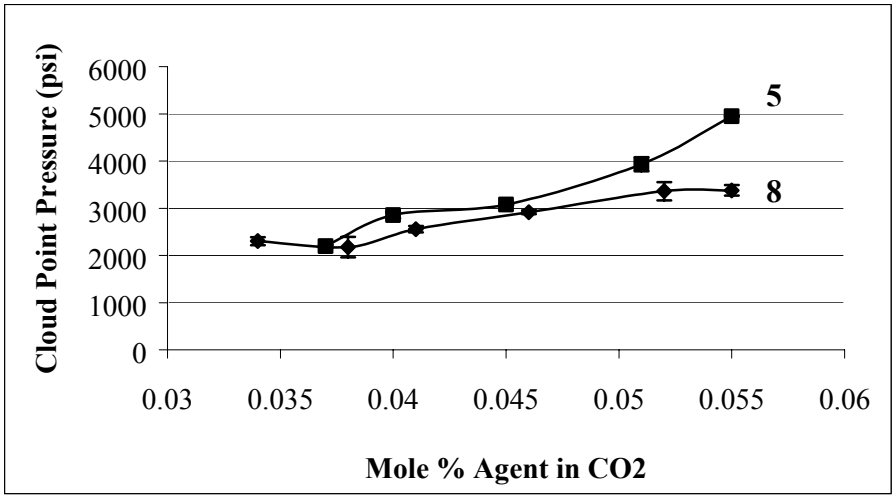
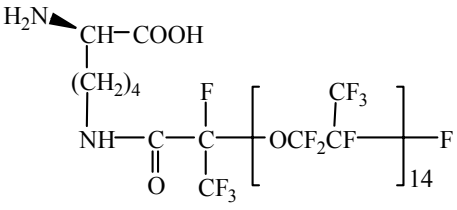
Figure 35 Comparison of Tail Length for L-Lysine Methyl Ester Derivatives





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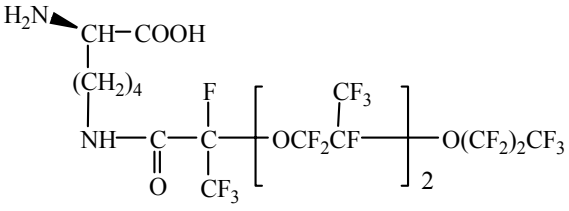

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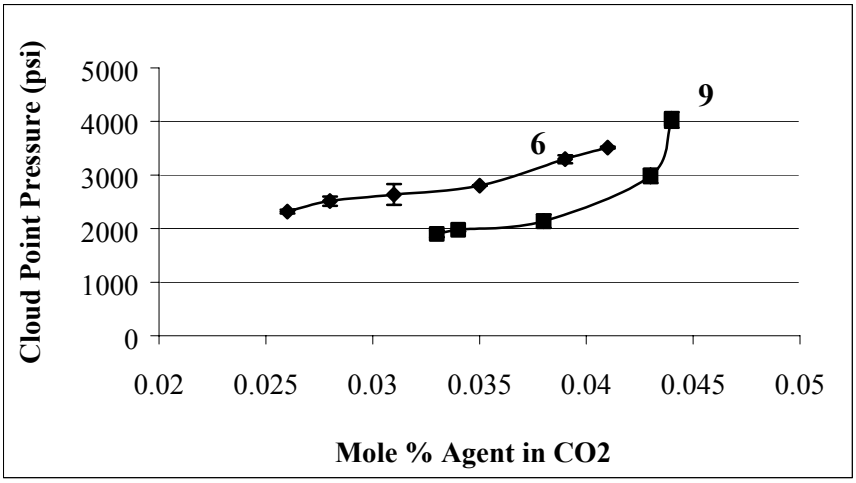
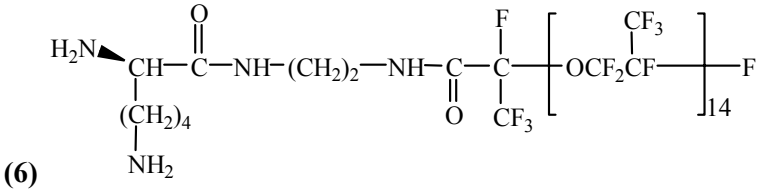
Figure 36 Comparison of Tail Length for L-Lysine Free Acid Derivatives





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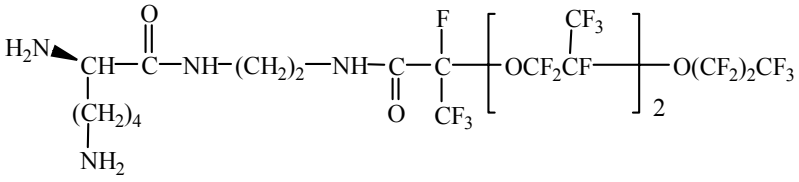


(9)

Figure 37 Comparison of Tail Length for L-Lysine Diamide Derivatives

Tail Placement. The next step was to investigate if tail placement affected the solubility of these agents in carbon dioxide. Comparing structures **4, 5,** and **6** in Figure 38, significant deviation in phase behavior is observed with molecule **6**. This molecule has two unique features as compared to molecules **4** and **5** in this family of agents which include; 1) two free primary

amines and 2) a two carbon spacer resulting in an increase in the molecular weight and polarity of the agent.

The existence of the two free primary amines presents the potential of carbamate and self association through hydrogen bond formation in high pressure CO_2. In addition, two more hydrogen bonding localities (two amides) have been incorporated into the molecule. These factors could possibly make a substantial contribution to the observed phase behavior. By increasing the potential for solute-solute interactions via hydrogen bonding, coupled with unfavorable solvent-solute interactions via carbamate formation, the observed pattern in phase behavior is reasonable for this molecule.

Intermolecular interactions of the α and ε amines of the lysine head group with the carbonyl groups would be strongly influenced by the pKa of the amines. The pKa of the α amine of the natural amino acid is referenced as 9.2 and that of the ε amine as 10.8. [72] The higher the basicity of the amine, the greater its affinity for a Lewis acid counterpart. Thus, it is anticipated that the ε amine would not only participate to a greater extent in solute intermolecular interactions than the α amine, but also in carbamate formation which is observed between structures **4** and **5** in Figure 38. In this case, heavy entropic and enthalpic penalties are paid in spite of the presence of a highly "CO_2 philic" tail. The lysine head group, though its contribution to the total molecular weight is small, dominates the molecule's phase behavior due to its probable interactions with itself and the surrounding medium.

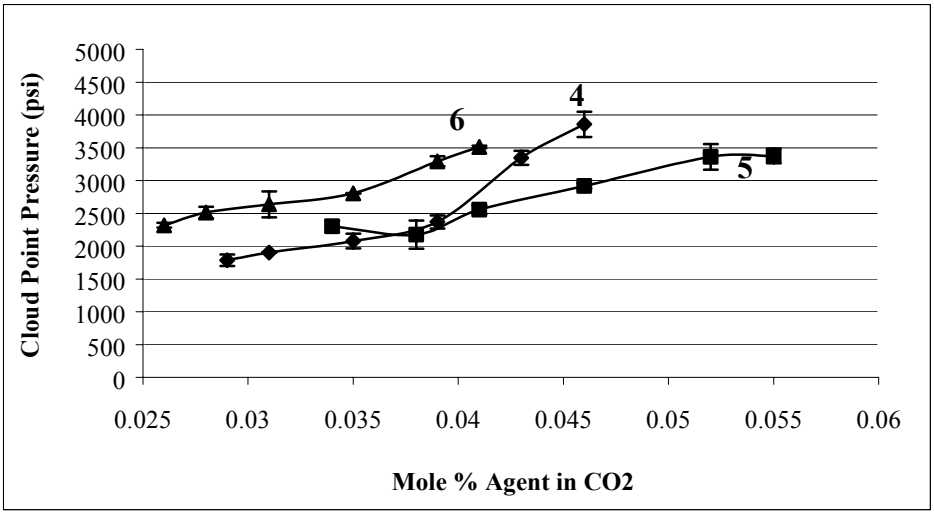
Examination of structures **4** and **5** in Figure 38 reveals a deviation in phase behavior as the concentration of the species increases. Comparison of the two molecules shows that structure **4** possesses an ester functional group and structure **5** a carboxy functional group. Because carbonyl functionalities have been shown to favorably interact with CO_2, it is

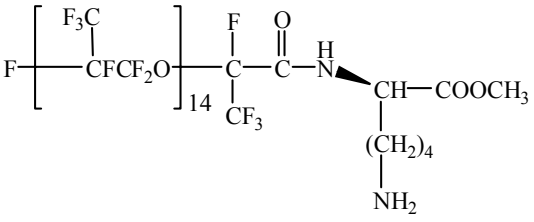
anticipated that structure **4** would exhibit a lower cloud point curve than structure **5**. Instead, the reverse trend is observed experimentally. As rationalized above, structure **4** has the greater potential to form intermolecular associations. It can be postulated that as the concentration of agent increases, larger intermolecularly attached complexes are formed, resulting in an increase in the macroscopic weight of the agent. This effect results in a decrease in solubility due to the formation of an entropically unfavorable complex. However, as will be described next, this behavior is not consistent with its other analogues. Thus, the reason why this effect is observed is unknown.

A comparison of the analogous molecules **7** and **8** in Figure 39 shows a different result. The cloud point curves exhibited by molecules follow the expected phase behavior pattern. In comparison to molecule **8**, molecule **7** possesses a greater number of CO_2 philic sites (ester versus carboxy acid), thus, promoting more favorable solute-solvent interactions and improved solubility. The primary structural difference between the **4** and **7** and **5** and **8** pairs is the fluoro tail attached to the lysine head group. As stated previously, the tail containing 2 repeat units imparted better solubility than the fluoro tail with 14 repeat units. As expected, entropic forces obviously predominate due to the large molecular weight increase, but do not fully explain why molecules **4** and **7** exhibit deviant behavior. Also in Figure 39, the same relative phase behavior for the diamine analogue as a function of tail placement for structure **9** is observed as that for molecule **6** in Figure 38.

The last series of fluorinated L-lysine derivatives to be investigated are those that have been modified with an eight carbon, fluoroalkyl tail. From phase behavior measurements made in our laboratories, it has been found that fluoroalkyl tails exhibit significantly less solubility than either tails composed of silicone or fluoroether functional groups. This phenomenon is

confirmed in Figure 40. Of the three modified molecules, possessing the same head group as those in Figures 38 and 39, only one agent was soluble in CO_2 (within the pressure limits of the testing apparatus – 8,000 psi). This agent, molecule **10**, exhibits the least solubility of the three tails and possesses the greatest similarity to a hydrocarbon, which is approximately 3 times less soluble in carbon dioxide than the fluoro – substituted analog. [52] The influence of the chemical properties of the head group on molecules **11** and **12** are important enough to render these molecules insoluble in carbon dioxide. The fluoroalkyl tail does not impart enough of a favorable enthalpic benefit to solubilize these molecules.





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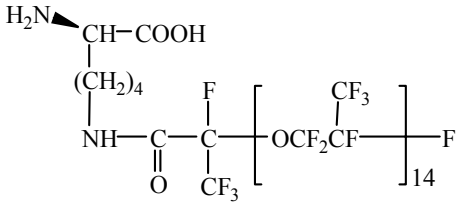

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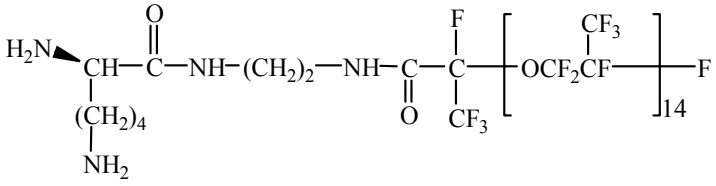
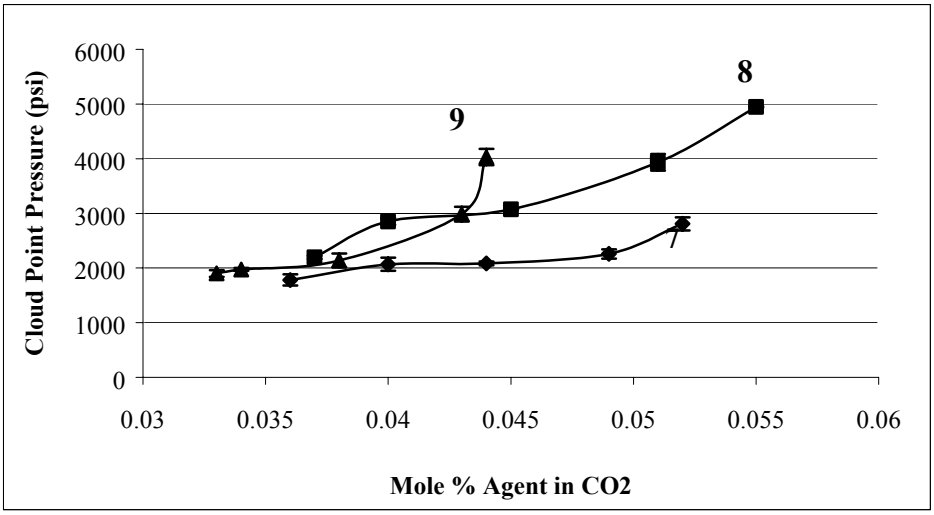
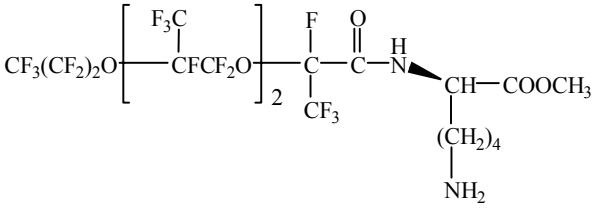


(6)

Figure 38 PHFPO L-Lysine Derivatives





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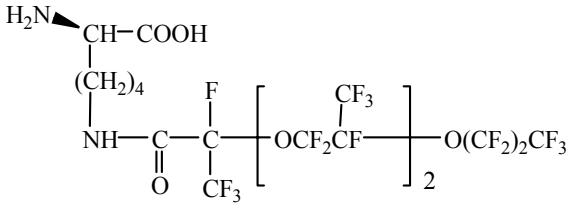

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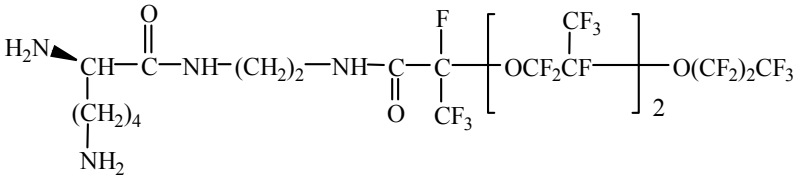

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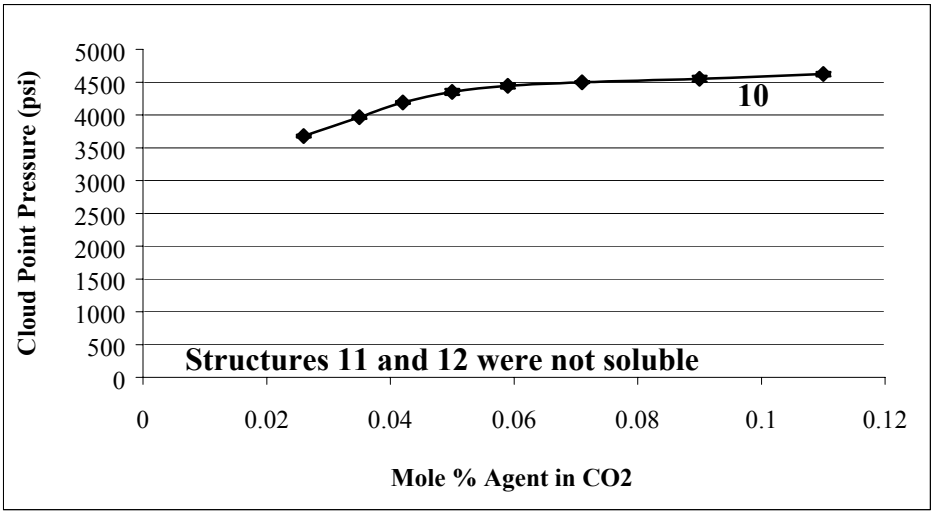
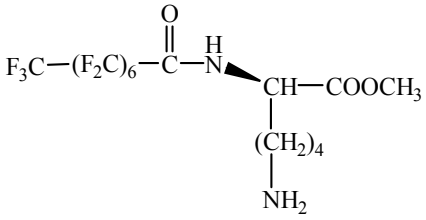
Figure 39 PTTF L-Lysine Derivatives





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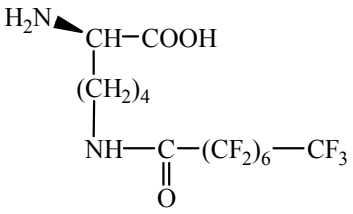

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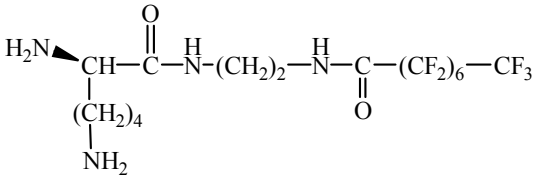

(12)

Figure 40 Fluoroalkyl L-Lysine Derivatives

7.4 General Phase Behavior of L-Lysine and Quinine Resolving Agent Systems

Examination of the previous experimental solubility data shows a characteristic pattern of phase behavior for solid-supercritical fluid systems. Though the agents, ibuprofen, and carbon dioxide show marked differences in molecular architecture, polarity, and molecular weight, the same broad phase behavior is observed. Below in Figure 41 is shown a general phase behavior diagram for the resolving agent systems evaluated in Figures 34, 38, 39, and 40.

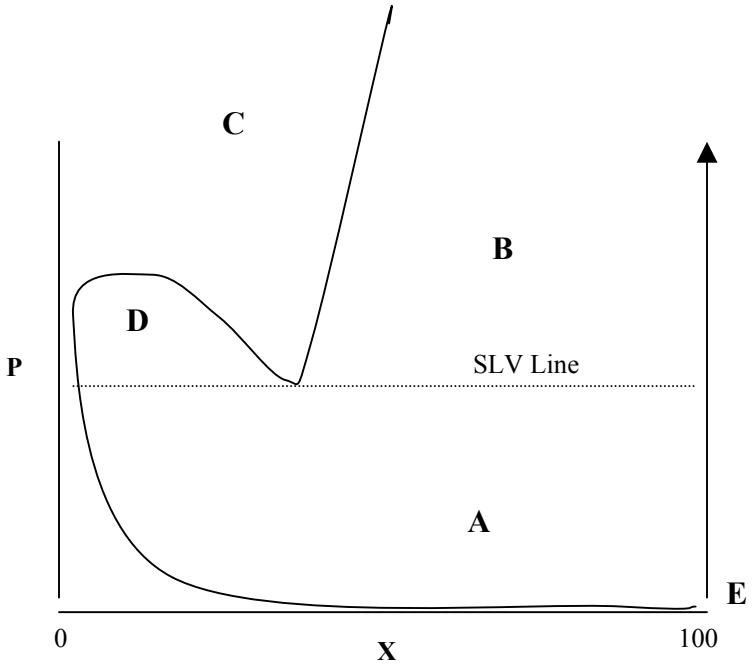

Figure 41 Phase Behavior for Solid-CO_2 Systems for $T > T_c$ of CO_2

The temperature at which the phase behavior of these resolving agent derivatives were tested was 30-32 ^{o}C (Controller temperature was set to 31 ^{o}C ± 1 ^{o}C). This temperature range represents a point approximately at the critical temperature of carbon dioxide. As a result, the following P-x relationship at constant temperature occurs and is described in greater detail below.

A : Vapor-Pure Solid Equilibrium

B: Fluid-Pure Solid Equilibrium

C: Single Phase Fluid

D: Vapor-Fluid Equilibrium

E: Vapor Pressure of Pure Solid

At pressures significantly below the critical pressure of CO_2, an equilibrium between pure solid and carbon dioxide vapor exists. As the system pressure is increased, the SLV (pure solid/liquid solution/vapor) line is intersected, which is descriptive of a three phase equilibrium, and forms a boundary between regions **B** and **A**, and **D** and **A**. Since the operating temperature is slightly below the critical pressure of CO_2, the SLV line is observed to occur at pressures that are slightly below the extrapolated vapor pressure of pure CO_2 at that temperature.

If the overall mixture composition is greater than the liquid phase composition, then by increasing the pressure an equilibrium between a fluid solution and pure solid exists. This is indicated in Figure 41 as region **B**. If, however, the overall mixture composition is less than the liquid compostition, than an equilibrium between nearly pure CO_2 and a fluid solution results as indicated by region **D**. One critical point also exists in this region. The highest pressure point on

the fluid-vapor envelope is the fluid-vapor critical point. Finally, region **C** in Figure 41 represents a region where a single phase fluid solution would exist.

The resolving agents investigated in this research project exhibited very low solubility, therefore, the mole % of agent in CO_2 is small and phase behavior characteristic of the dilute solution range is expected. Thus, fluid-pure solid equilibrium phase behavior will not be observed. In addition, the concentration of solids in this region is most likely dilute enough, so that the physical effects, such as melting point depression, will also not be observed. [36,80]

One important experimental observation requires mention. Upon depressurization, precipitation of solute is evident at higher pressure than visual bubble formation. This observation suggests that the fluid-vapor envelope is small and the fluid-vapor critical point cannot be accurately measured by the current system.

7.5 Conclusion

The use of natural products for pharmaceutical processing in a supercritical carbon dioxide medium presents a substantial challenge due to the poor ability of carbon dioxide to solubilize polar materials. Derivatization of these materials presents an avenue with which to move these insoluble materials into an environmentally benign reaction/processing environment. Careful consideration must be given to how and where a native molecule is derivatized and, ultimately, how will it affect the reaction process.

Thermodynamic mixing rules describe how a derivatized molecule will behave and finding a balance between entropic and enthalpic effects dictates how the molecular architecture

of the molecule is to be constructed. In summary, functional groups which exhibit low cohesive energy density and do not self associate are well suited for the CO_2-philic portion of the molecule. Unfortunately, hydrogen bonding functional groups are required at the head group in order to bind to the target ibuprofen molecule. Minimization of hydrogen bonding functionalities in the head group which are not participating in complex formation improves the likelihood of greater agent solubility.

The next step in this investigation is to test the ability of these molecules to form ion pairs and again how these molecules collectively behave in a supercritical carbon dioxide environment.

8.0 MEASUREMENT OF EQUILIBRIUM CONSTANTS IN CO_2

8.1 Introduction

In order to resolve the enantiomers of ibuprofen, the thermodynamic equilibrium resulting from the formation and stability of the salt complexes in a saturated carbon dioxide medium requires evaluation. The relative value of the equilibrium constant will presumably indicate to what extent salt formation occurred and whether selectivity of the resolving agent has been preserved compared to its selectivity in organic solution. Based upon these observations, the resultant salts formed from the complexation of ibuprofen and resolving agent in carbon dioxide will either be:

- separated as a function of phase behavior differences in P-x space and the natural affinity of the resolving agent for each enantiomer of ibuprofen.
- separated only as a function of their phase behavior differences in P-x space.
- inseparable due to the formation of eutectics, loss of selectivity of the resolving agent, and/or possessing similar phase behavior in P-x space.

Highly selective agents are expected to yield better separation results owing to the greater difference in solubility between free ibuprofen and unbound resolving agent as opposed to a diastereomeric salt pair. In addition, higher selectivity also implies that an excess of one enantiomer of ibuprofen will be present. The separation of bound ibuprofen from unbound ibuprofen depends then not only upon the reactivity of each species and their resultant

concentration in solution, but also on their collective phase behavior. The reactivity of each species describes how much of each species will form, thus indicating how much of each enantiomer will remain unbound.

The greater the disparity in phase behavior between unbound agent, salt complex, and unbound ibuprofen, the likelihood of a better separation. Ideally, if an agent retains its selectivity in CO_2, an efficient separation process may be constructed. As discussed in Chapter 7, the phase behavior of the modified resolving agents varied as a function of the "CO_2 philic" tail that was attached to the natural agent. The agents possessing fluoroether tails exhibited miscibility pressures well below that of free ibuprofen. Conversely, those agents which possessed a fluoroalkyl tail exhibited miscibility pressures well above that of ibuprofen. The phase behavior of the resultant salts formed from these modified agents is also anticipated to be heavily influenced by the nature of the tail attached to the resolving agent. For example, those modified agents which are marginally soluble in CO_2 are intuitively not expected to produce a more soluble salt complex. Likewise, those agents that exhibit higher solubility are more likely to produce soluble salt complexes.

Basing the selectivity of the resolving agents on their behavior in organic media, however, cannot be applied in a direct manner to supercritical carbon dioxide. Not only are separations of this type solvent dependent, but also are usually performed in the presence of a polar component, whether it is the solvent itself or a modifier. Due to the extreme non polar nature of CO_2, deviations from currently reported literature data are anticipated. In addition, chemically modified agents will be used, and these modifications have the potential to affect the binding capacity of the resolving agents thereby reducing the expected selectivity of the resolving agent.

8.2 Ion Pair Formation and Equilibrium

As described previously, the enantiomers of ibuprofen are industrially resolved using diastereomeric crystallization. Thus, the formation of ion pairs and their resultant solubility is the primary mechanism by which separation occurs. This mechanism is verified from crystallographic studies of ibuprofen lysinate complexes. From these studies, it was found that a hydrogen bond is formed between the carboxylic acid of ibuprofen and the ε amine of lysine. [55, 56, 57, 58, 59, 60,61] The comparison then between the known industrial method and the method using CO_2 as proposed in this research project is highly dependent upon the reactivity of these molecules in their respective solvents. This becomes primarily a comparison between an ethanol/H_2O solution, which is used industrially, and carbon dioxide.

Application of diastereomeric crystallization in high pressure carbon dioxide is presented schematically in Figure 42. Initially ibuprofen (shown as one enantiomer in this schematic) and resolving agent are present as free species in the solid phase, which is represented as the area under the straight line in the schematic. Upon pressurization of the CO_2 fluid phase, resolving agent and ibuprofen begin to dissolve into the CO_2 bulk fluid phase and react to form an ion pair complex. Dissolution and reaction in this schematic are not meant to imply that these events happen separately, but probably occur simultaneously. In the bulk fluid phase, there will be at least three species present (or five if both enantiomers are present). As discussed in Chapter 7, the solvation of higher molecular weight species is entropically disfavored and may potentially result in the precipitation of that particular species from solution. Precipitation is a physical process in which a phase change occurs so that the reaction solution becomes a two phase system. Most precipitations begin slowly, accelerate, and then slow down again. [85] If the salt

were to precipitate from the bulk phase, more salt will form according to LeChatelier's principle, resulting in a dynamic process with the quantity of salt precipitated dependent upon time. Finally, after an infinite amount of time, all resolving agent and ibuprofen will be bound and precipitated from solution.

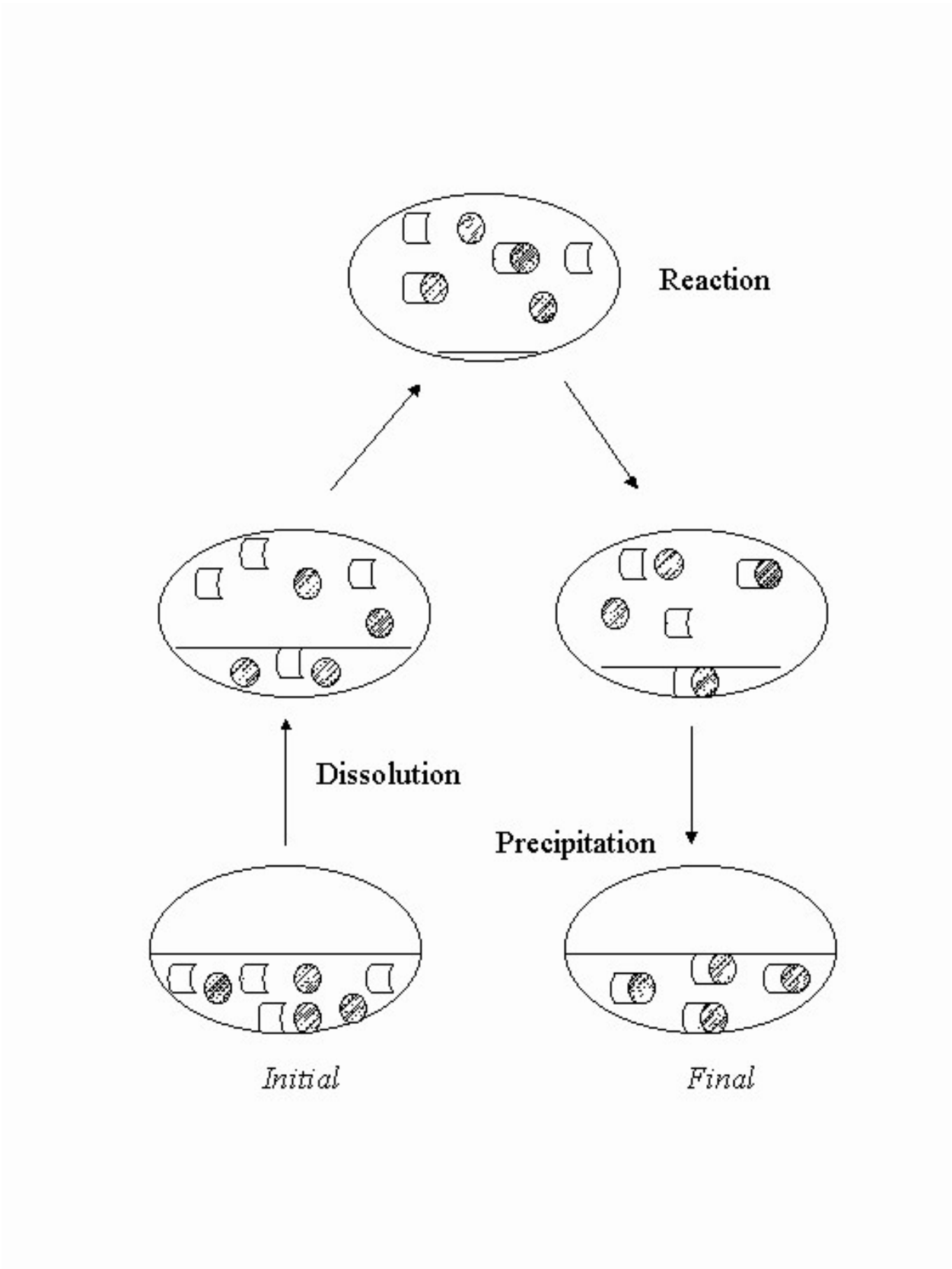

Figure 42 Diastereomeric Crystallization in High Pressure CO_2

The association or dissociation of ions to form ion pairs is dependent upon the polarity of the solvent in which they exist. For example, in solvents of low polarity ions tend to associate themselves into pairs, and this affinity of the cation for the anion is also an observed solvent dependent phenomenon. [18,81] These ion pairs may exist in two distinct forms, as either the direct contact or solvent separated form. In the direct contact form, no solvent molecules are spatially located between the two species, while a solvent separated pair has a solvent molecule separating the two. Both of these ion pair types may be detected spectroscopically. [79]

Whether direct contact or solvent separated, the interaction between the resolving agent and ibuprofen is based upon the reaction of an acid and a base. Though the reaction takes place in a non polar medium, the stability of that resultant ion pair may not be favorable. Therefore, equilibrium measurements are necessary to evaluate whether CO_2 serves as a suitable medium for reactions of this type.

A general equilibrium model is employed in an attempt to determine the predominant observable equilibrium reaction. Reaction systems of this type exhibit many equilibria, including those for dissolution, main reaction, side reactions, nucleation, and precipitation. Kinetic effects may also be present, particularly with those processes that are typically slow to reach equilibrium, such as precipitation processes. The current experimental setup does not allow for the identification and measurement of any one single equilibrium. Instead, the dominant equilibrium is measured and a simple model is derived in order to correlate these measured effects. The following assumptions are used for the construction of a simple model equilibrium equation.

- It is assumed that the observed equilibrium is readily established based upon the nature of the interaction between ibuprofen and the resolving agent. The association

formed from this interaction is likely an ion pair possessing a hydrogen bond. [55, 56, 57, 58, 59, 60, 61] Hydrogen bonding, functioning as the sole attractive force, has been shown to be sufficient for optical resolution using liquid chromatography. [86] Hydrogen bonds are also known in solution to rapidly form and dissociate. For example, the mean lifetime of such a bond is on the order of 10^{-12}seconds. [82] Thus, ion pair formation is expected to occur rapidly and equilibrium readily established within the designated experimental time frame of two hours.

- The molar ratio of total ibuprofen to resolving agent that will be used in these experiments is 1:1. Thus, for every mole of ibuprofen there is one mole of agent available for complexation.
- Dimerization of ibuprofen is insignificant.
- The rate of precipitation, if it occurs, is assumed to be significantly less than either the rate of dissolution or the rate of ion pair formation. As noted by Freiser and Fernando, a slow equilibration of solid and solute exists during precipitation phenomena, the duration dependent upon the nature of the solute and solvent. [80]

The equilibrium expression of one mole of an enantiomer of ibuprofen reacting with one mole of resolving agent may be written as

$$[R]+[I] \underset{\leftarrow}{\longrightarrow} [Salt]_{sol} \underset{\leftarrow}{\longrightarrow} SP \qquad (8\text{-}1)$$

where [R] is the concentration of resolving agent, [I] is the concentration of ibuprofen, $[Salt]_{sol}$ is the concentration of salt in solution, and SP is the number of moles of salt precipitated from solution. Thus, the equilibrium constant is expressed as follows.

$$K_{eq} = \frac{[Salt]_{sol}}{[R][I]} \tag{8-2}$$

The total amount of salt produced at a given time is shown by the following mass balance,

$$V * [Salt]_{sol} + SP = V * [I]_o - V * [I] \tag{8-3}$$

where $[I]_o$ is the initial concentration of ibuprofen and V the reactor volume.

The mass balance expression for one mole of ibuprofen binding to one mole of resolving agent is

$$V * [I]_o - V * [I] = V * [R]_o - V * [R] \tag{8-4}$$

and dividing by the volume and rearranging, Equation (8-4) becomes

$$[R] = [R]_o - [I]_o + [I] \tag{8-5}$$

Substituting in Equation (8-2) with the expression for$[Salt]_{sol}$ from Equation (8-3), and dividing by the volume Equation (8-2) becomes

$$K_{eq}[R][I] + SP/V = [I]_o - [I] \tag{8-6}$$

Substituting Equation (8-5) into Equation (8-6) and assuming $[I]_o = [R]_o$

$$K_{eq}[I]^2 + [I] - [I]_o + SP/V = 0 \tag{8-7}$$

If no precipitation occurs or the rate of precipitation is significantly slow relative to the measurement of equilibrium then the equilibrium expression reduces to

$$K_{eq} = \frac{[I]_o - [I]}{[I]^2} \tag{8-8}$$

If precipitation is significant, then the equilibrium expression becomes

$$K_{eq} = \frac{[I]_o - [I] - SP/V}{[I]^2} \tag{8-9}$$

and equations (8-8) and (8-9) represent the upper and lower boundaries of the equilibrium constant. In this series of experiments precipitation was not visually observed. This implies that either precipitation did not occur or the rate of precipitation is significantly slow. In either case, the SP/V term in Equation (8-9) is assumed negligible and the model equilibrium equation reduces to Equation (8-8).

8.2 Experimental

The quantity of free ibuprofen versus bound ibuprofen was measured by an indirect method. This method was developed as an alternate to direct *in situ* measurements, since a high pressure UV or IR apparatus was not available. A qualitative experiment was designed to determine equilibrium constants to a rough order of magnitude. The results of these experiments are shown in Figures 43 to 52. Equilibrium constants for each individual enantiomer of ibuprofen were measured independently from one another. The purpose of designing the experiment in this manner was to be able to:

- Distinguish differences in equilibration time between the two enantiomers and the resolving agent.

- Distinguish differences in the selectivity of the resolving agent for the two enantiomers of ibuprofen.

Typically, a 1:1 mole ratio of the resolving agent to either enantiomer of ibuprofen is loaded into a high pressure cell, charged with CO_2 to a pressure of 5800 psi (which is a pressure above the phase boundary curves for each of the components), and vigorously stirred. Samples of the fluid phase are then taken at specified time intervals. To take a sample, a high pressure sampling value (Rheodyne, Inc.) is turned to the load position. To recover the sample, the value is manually switched and the sample is collected in a 5 ml n-hexane liquid trap and washed with additional 1ml n-hexane through the flush port (0.4 ml dead volume). Hexane was chosen because it is also a nonpolar medium, and ibuprofen is soluble in this medium while the native agents and the corresponding salt complexes are not. Thus, the equilibrium quantities of free and unbound species are expected to remain similar and what material is collected in the hexane phase is composed primarily of unbound ibuprofen.

The sample is then filtered (Millipore) to remove solid particulates and the solvent evaporated. The solid sample is then redissolved in the HPLC mobile phase for analysis. The flush port on the reactor is then dried with an air stream to ensure that all solvent has been removed from the sample loop prior to taking the next sample.

The samples were analyzed for unbound ibuprofen by a Hewlett Packard Series II 1090 HPLC unit using a Chiracil-ODH (Chiral Technologies) chiral column. The mobile phase consists of 98% hexane: 1.9% 2-propanol: 0.1% TFA, and the analysis was run with a flow rate of 0.8ml/min at 38°C. The run time for sample analysis was 8 minutes. The concentration of each enantiomer was obtained by comparing the peak area from the HPLC chromatogram

against a standard curve, and the equilibrium constant was calculated according to Equation (8-8).

Bound species are also assumed not to significantly dissociate due to the observation that ions will aggregate in non polar media. This assumption was verified for the salt complexes in carbon dioxide. In this experiment, the salt complexes were formed prior to introduction into the batch reactor. The cell was pressurized to 7000 psi and gently stirred for 2 hours, representative of the time allotted for equilibrium measurements. The stirring was stopped for 30 minutes and samples of the fluid phase were taken and analyzed by HPLC. The same analytical procedure was employed except that TFA was omitted from the mobile phase. The total ibuprofen content was measured and results indicated that a maximum of 5% dissociation occurred for most of the salts.

8.3 Results and Discussion

8.3.1 The Quinine Family

The equilibrium data for the quinine series of resolving agents is presented in Figures 43, 44, and 45. The measured equilibrium constant, K_{eq}, is interpreted to be reflective of not only the amount of unbound resolving agent present in solution as a function of time, but also identifies which enantiomer would be present in excess. As stated previously, the selectivity of the agent will depend in part not only upon the structural architecture of the resolving agent, but also the

medium in which it functions. Inherent changes in the agent's selectivity are anticipated due to the structural modifications made to the agents in these experiments.

In order to present a general explanation for the behavior of the measured K_{eq}, the following assumptions and experimentally measured parameters are employed. These statements are applied to all families of resolving agents used in this series of experiments.

- Ibuprofen is soluble at the specified operating conditions as shown by Khundker and coworkers and experimentally verified. [29]
- The resolving agents are soluble at the specified operating conditions as was shown in Chapter 7.
- It is initially assumed that the dominant equilibrium reaction is the formation of an ion pair.
- Since precipitation was not visually observed in these experiments, Equation (8-8) is employed to calculate K_{eq}.
- The formed salt complexes undergo minimal dissociation into its native starting materials as previously described in the experimental section.

Comparing the data from Figures 43, 44, and 45 two distinct trends are observed. First, it is evident by comparing the trend lines in these graphs that the relative value of the equilibrium constant appears to stabilize in the range of 5 to 30 minutes. Faster equilibration times are observed with the resolving agents that exhibit higher solubility, i.e. silicone functionalized, at the operating conditions. It may be inferred in this case that the time required to reach the observed equilibrium is highly dependent upon the rate at which the agent dissolves into the bulk

CO_2 phase. Here, the forward and reverse reaction rates of solution present another potential equilibrium reaction.

Also shown in Figures 43, 44, and 45 is the relatively large values for K_{eq} within the first 15 minutes of the experiment. According to Equation (8-8), a large value of K_{eq} is reflective of a large concentration of ibuprofen present as its complexed salt. This particular effect observed in the graphs, however, is not attributed to salt formation in the initial time period of the experiment. A more plausible explanation, rather, is that during this time period the dissolution of ibuprofen into the bulk CO_2 phase is still occurring. An artificially low free acid concentration would be measured, thus producing a large value for K_{eq}. Therefore, Equation (8-8) cannot be properly applied to this initial phase since other physical processes, for example, solubilization of reactants and their associated rates, appear predominant.

If precipitation of the salt complex were to occur, the equilibrium model used for this reaction system would produce overestimated values of K_{eq} due to the exclusion of the precipitate term. During experimentation, however, no visible precipitate was observed. This implies that either precipitation did not occur to any noticeable extent during the time period of the experiment, the solution was dilute enough so that no visible precipitation would be visually seen, or that salt did not form. The later is ruled out simply because variations in K_{eq} occurred with time.

If precipitation were occurring at a significant rate, than a decrease in K_{eq} as a function of time would be expected. As shown in Figures 43, 44, and 45 this effect is not observed and instead a consistent value of K_{eq} is shown, supporting the assumption that the rate of precipitation is considerably much slower than the relative measurement of equilibrium. According to Freiser and Fernando and Nielsen there exists a slow equilibration of solid and

solute during precipitation phenomena, with the rate of precipitation characterized by a period of induction. [80, 85] During this process, nucleation is occurring, and the rate at which this process occurs is concentration and solubility dependent. Generally, the higher the concentration of salt formed and the less soluble in its solvent medium, the faster the rate of precipitation. [85] For this qualitative analysis, then, these anticipated heterogeneous systems may be then treated as homogeneous since precipitation phenomena does not appear to provide a significant contribution to the measurement of K_{eq}.

The silicone functionalized quinine resolving agent as shown in Figure 44 also exhibits a stabilization of K_{eq} over time. It appears with this resolving agent that equilibration occurs much faster as compared to the fluoroalkylated agents. The rate of solubilization of this agent is likely much faster as compared to the fluoroalkylated agents due to the much higher solubility of the silicone derivatized resolving agent in CO_2 as shown in Figure 34 of Chapter 7. In addition, at the conclusion of the experiment where t=120 minutes, the average value for K_{eq} for the silicone based agent was higher than that for the fluoroalkyl based agent, indicating that this particular agent was able to more efficiently bind ibuprofen.

Interestingly, the fluoroalkylated quinidine resolving agent produced measured equilibrium constants one order of magnitude higher than either the fluoroalkyl or silicone functionalized agents with its apparent time to reach equilibrium consistent with the fluoroalkyl derivatized quinine. According to this data, the quinidine analogue performed superior in its ability to bind ibuprofen in comparison to the other two agents. Structurally, the agents in this family exhibit significant differences not only by tail character and placement, but also stereochemically. Quinidine is the chiral inverse of quinine. These results emphasize the

importance of resolving agent architecture in terms of the ability of a bulky molecule, such as ibuprofen, to efficiently bind to the modified agent and maintain a stable salt complex.

One other point to note is the large error associated with the experimental results. This is in part attributed to the method of analysis and the experimental setup. In terms of the method of analysis, the analytical method used to measure the concentration of ibuprofen does so on a milligram scale and large differences in concentration may result from small changes in the quantity of ibuprofen collected. For example, upon examining the average K_{eq} for the three different agents in this family, there is almost an order of magnitude difference. The actual quantity of ibuprofen loaded into the cell differs by 0.01g between the three sets of experiments. Optimally, an *in situ* analytical technique would preserve mass in the analysis, however, a high pressure on-line instrument was not available.

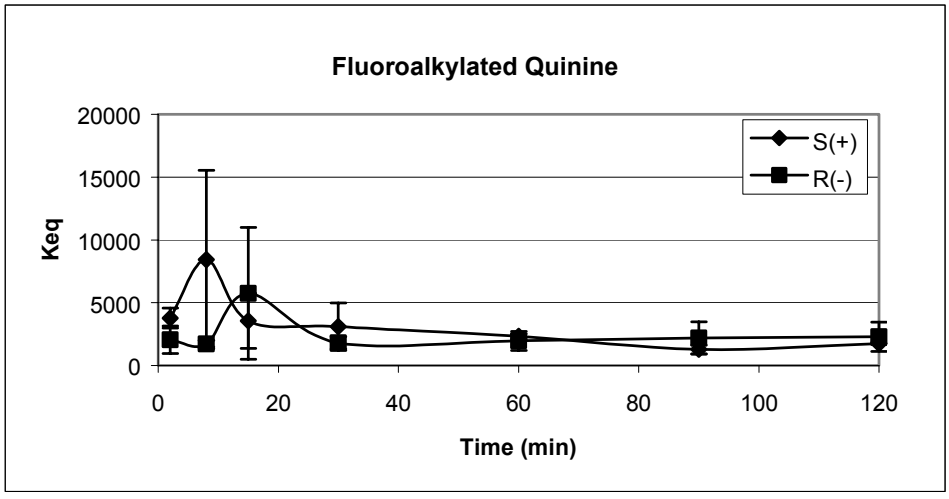

Figure 43 Equilibrium Data for the Fluoroalkyl Functionalized Quinine

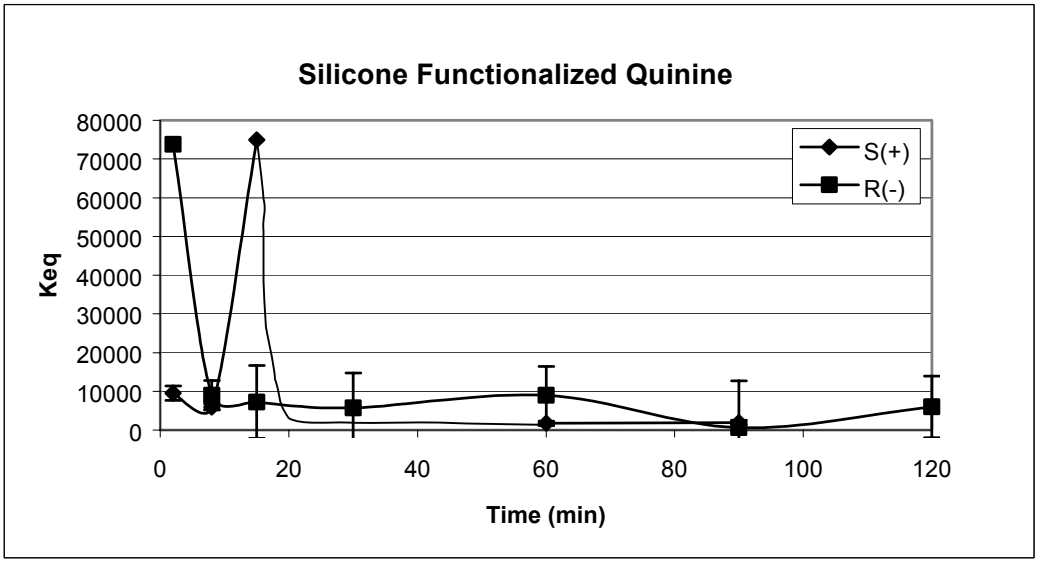

Figure 44 Equilibrium Data for the Silicone Functionalized Quinine

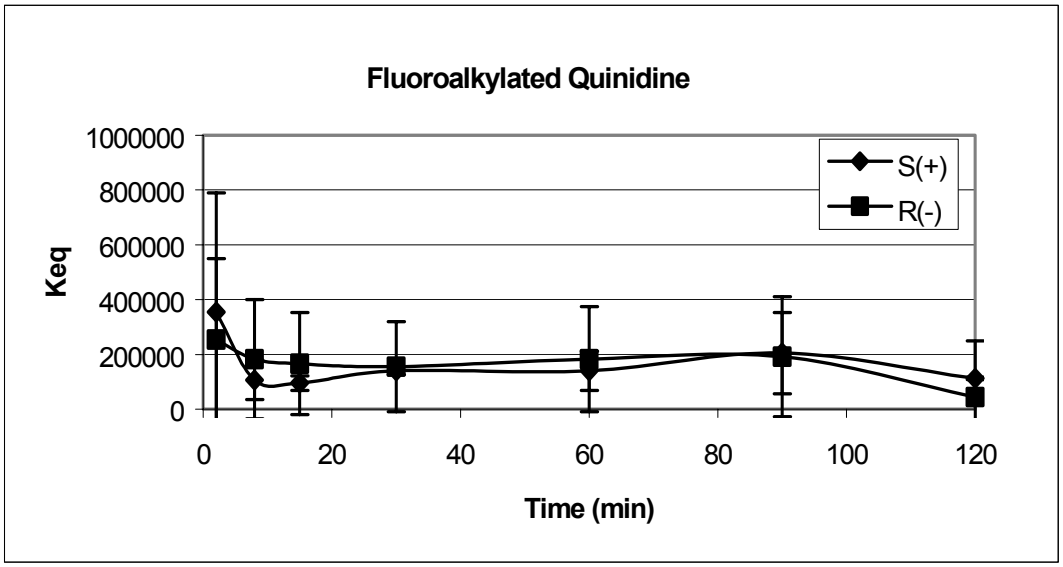

Figure 45 Equilibrium Data for the Fluoroalkyl Functionalized Quinidine

The second observed trend concerns the selectivity of the agent. As shown from Figures 43, 44, and 45 only the fluoroalkyl functionalized quinine exhibited any degree of selectivity among the three resolving agents in this family, though this difference in K_{eq} remains relatively small. This experimental result is contrary to molecular modeling results presented in Chapter 4. From the theoretical calculations, it was predicted that the silicone functionalized quinine would retain the greatest degree of selectivity. As compared to the fluoroalkyl analogue, the silicone tail was placed at a position removed from the chiral secondary alcohol. This alcohol group functions as a source of hydrogen bonding, which is a critical point for chiral discrimination. The fluoroalkylated analogue, however, was derivatized at the chiral secondary alcohol to form an ester linkage and, thus, theoretically reduced its ability to hydrogen bond.

The fluoroalkyl functionalized quinidine, as shown in Figure 45, exhibited no selectivity. This result is consistent with molecular modeling studies presented in Chapter 4. As compared to quinine, not only are functional group and/or tail placement critical, but also the spatial configuration. As stated previously, complexation of ibuprofen's carboxylic acid group is assumed to occur at the amine located in the bicyclic portion of the molecule. Due to the bulkiness of the molecule, molecular motion is limited and thus the spatial configuration of those functional groups that participate in complexation through hydrogen bonding, for example, must be strictly defined for chiral discrimination to take place.

It cannot be concluded exclusively that the observed loss of selectivity of these agents is strictly attributed to chemical modification, but rather the solvent system may also play a significant role. The selectivity that was observed in a polar organic medium was not evidence in carbon dioxide, thus proving carbon dioxide an inferior substitute. The salt complexes formed

from these agents in CO_2, therefore, will have to be separated as a function of their phase behavior alone.

8.3.2 The Polyperfluoropropylene Oxide Derivatized L-lysine (Krytox) Family

The next series of equilibrium data involve resolving agents that contain L-lysine as their chiral discriminator. In this family, L-lysine has been derivatized with a polyperfluoroether tail containing 14 repeat units (MW ~ 2500). These agents possess the ability to form soluble salt complexes and, thus, a homogeneous reaction system. Any possibility of precipitation of the resultant salt complexes is not anticipated to occur due to the significantly higher solubility of the modified resolving agents as compared to those in the quinine family. The equilibrium data for the three agents in this family are shown in Figures 46, 47, and 48.

Contrary to that observed for the quinine family of agents, it can be clearly seen that equilibrium is established within 15 minutes for only one of the agents, namely the N'-polyperfluoroether agent shown in Figure 46. The other two agents, shown in Figures 47 and 48, exhibit inconsistent K_{eq} as a function of time for the entire experimental duration. The operating pressure of the system is well above that of the cloud point pressure for these agents. It is expected, then, that solubilization of the starting materials occurs quickly and equilibrium would be readily established.

Unlike the behavior exhibited by the quinine family of agents, this data shows an increase in K_{eq} as a function of time, until a maximum is reached and either stabilizes or slightly begins to decrease. This decrease in K_{eq}, though difficult to discern because of the large error involved, may be due to a slight dissociation of the salt complexes, which would introduce another kinetic

parameter to the reaction system. Unfortunately, these types of competing kinetic effects are difficult to discern from the present data, since the experimental setup was not designed to quantitatively evaluate individual kinetic mechanisms.

For the agents shown in Figures 47 and 48, the polyperfluoroether tail appears to significantly interfere with the binding capability of its head group. The polyperfluoroether tail is not only very flexible about its single bonds, but also significantly longer than any of the "CO_2" philic tails placed on the native agents. This tail has the ability to twist and wrap about the head group, which would be advantageous for solubilization. In other words, possessing the ability to mask the polar portion of a molecule with a "CO_2 philic" structure has the potential to improve and maintain solubility at lower pressures. This spatial configuration, assuming that it is the lowest energetic configuration, may make it difficult for ibuprofen to truly bind to the head group if a bulky tail obstructs its pathway. The agent shown in Figure 46, however, exhibits a stable K_{eq} as a function of time. The site of complexation for this agent would occur at the α amine, which is located adjacent to the chiral carbon and possibly less accessible to the flexible tail.

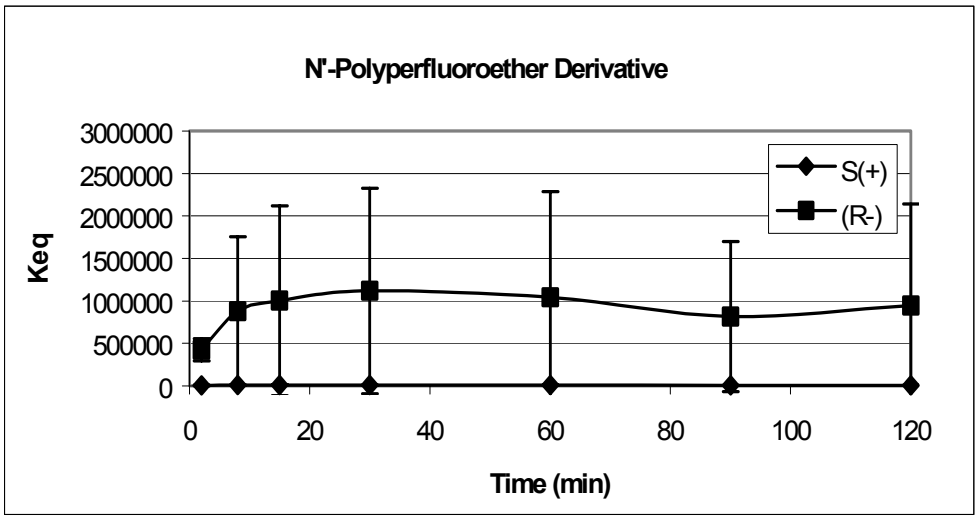

Figure 46 Equilibrium Data for N'-(Polyperfluoroether)-L-Lysine

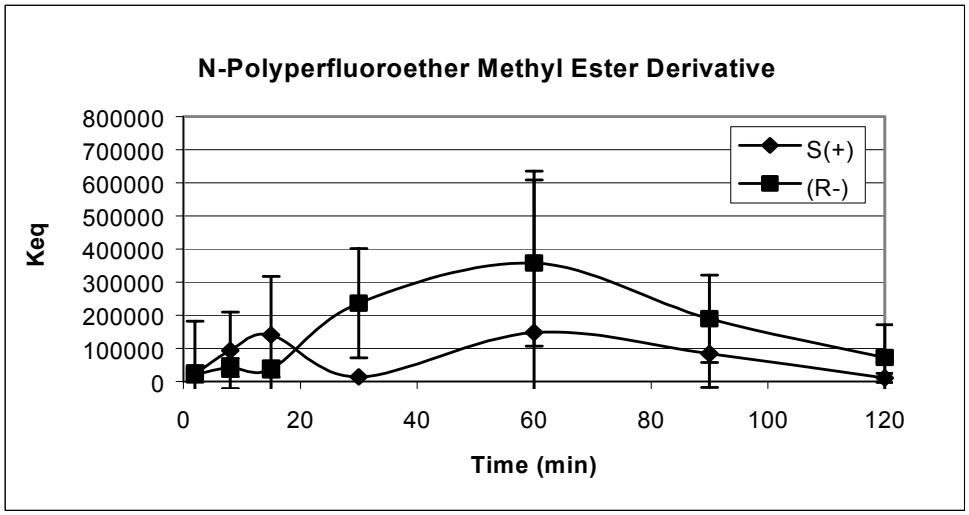

Figure 47 Equilibrium Data for N-(Polyperfluoroether)-L-Lysine Methyl Ester

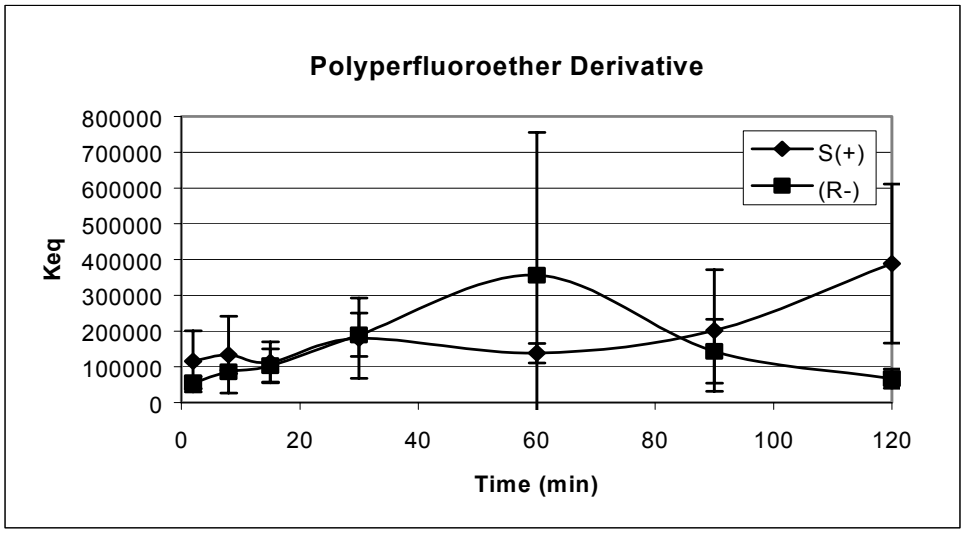

Figure 48 Equilibrium Data for (Polyperfluoroether)-L-Lysine With Alkyl Spacer

Interesting trends are observed for the selectivity of these agents. Examining Figures 46, 47, and 48 the average K_{eq} for each agent shows a consistent preference for the R(-) enantiomer. This is contrary to the molecular modeling studies as presented in Chapter 4. The theoretical calculations indicate that there exists a slight preference for the S(+) enantiomer and that when translated into enantiomeric excess, there should be no discernable selectivity for all of the three agents in this family as a whole. Molecular modeling results also predicted that the N-polyperfluoro methyl ester derivative would provide the greatest selectivity of the three agents, though this selectivity is minimal.

Evaluating the average trend line for K_{eq} in Figure 47, it is shown that not only does the selectivity decrease as a function of time, but also K_{eq}. A reduction in K_{eq} indicates that the concentration of free ibuprofen is increasing, leading to the belief that this particular agent is not effectively binding ibuprofen. The trend lines for the other two derivatives, as shown in Figures 46 and 48, indicate that a greater degree of selectivity is observed with these agents. The

polyperfluoroether derivative suggests that after 80 minutes, the agent exhibits a preference for the S(+) enantiomer, which is consistent with molecular modeling results. The N'-polyperfluoroether derivative in Figure 46, however, maintains a minimal preference for the R(-) enantiomer. Due to the large standard deviation calculated for this experimental set, however, it is difficult to discern if any selectivity exists.

From an operational standpoint, these agents would not be practical to use. Since the solubility of the agent as a whole is strongly dictated by the contribution of the tail, separation of the native agent and product would be difficult. As stated previously, the salt is also a soluble component of the reaction system and its solubility is also anticipated to be very similar to the derivatized agent. Without kinetic information, it is not obvious how separate factors influence the reaction itself. For example, it is not obvious how effectively ibuprofen binds to the agent itself or if and to what extent ibuprofen undergoes secondary reactions, such as dimerization.

8.3.3 The Perfluoro-2,5,8-trimethyl-3,6,9-trioxidedodecanoyl fluoride Derivatized Lysine (Lancaster) Family

Another family of agents that possess lysine as the chiral discriminator is presented in Figures 49, 50, and 51. In this family, lysine has been derivatized with a short chain perfluoroether tail (2 repeat units) at the same positions as the polyperfluoroether (Krytox) modified agents previously discussed. These agents also form soluble complexes, so a homogenous system results from the reaction of the resolving agents and ibuprofen.

Comparing the data from Figures 49, 50, and 51 an inconsistent trend is again observed with regards to the time required to reach equilibrium. For example, it may be noted that in

Figures 49 and 50, equilibrium has been reached at approximately 15 minutes. The trend line in Figure 51, however, is less discernable. To be able to distinguish whether the causes of these deviations are related primarily to a reaction system in which each species is highly soluble or related to the natural error of the sampling/measurement technique, a comparison to the previous polyperfluoroether (Krytox) based analogues may be made. For example, the inability to show a relatively constant K_{eq} with time is a feature that both agents have in common. However, this appears to be inconsistent within analogues. For example, the N'-(polyperfluoroether)-L-lysine (Krytox) derivative was the one agent in that family that appeared to establish equilibrium. In this family of agents (Lancaster), however, both the N-(perfluoroether)-methyl ester and N'-(perfluoroether) derivatives appeared relatively stable. Interestingly, the perfluoroether derivative of both families failed to establish equilibrium and the same cross over of the trend lines is evidenced. However, the perfluoroether (Lancaster) agent exhibited a preference for the R(-) enantiomer instead of the S(+). Though a lack of consistency is shown between both families of perfluorinated modified agents, the trend lines exhibit a similar pattern.

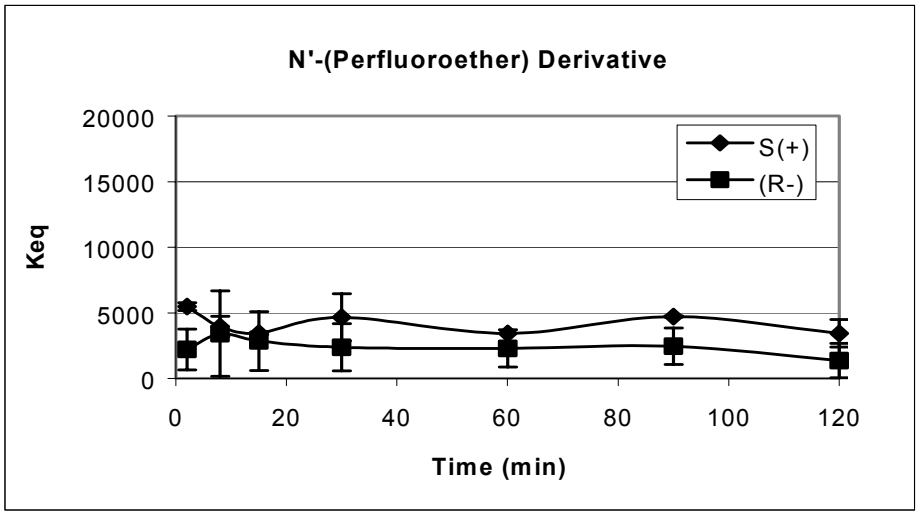

Figure 49 Equilibrium Data for N'(Perfluoroether)-L-Lysine

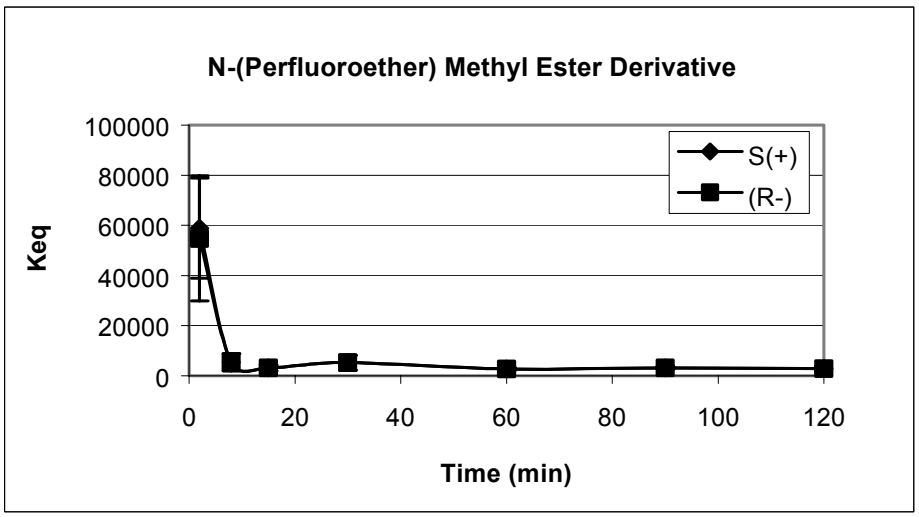

Figure 50 Equilibrium Data for N-(Perfluoroether)-L-Lysine Methyl Ester

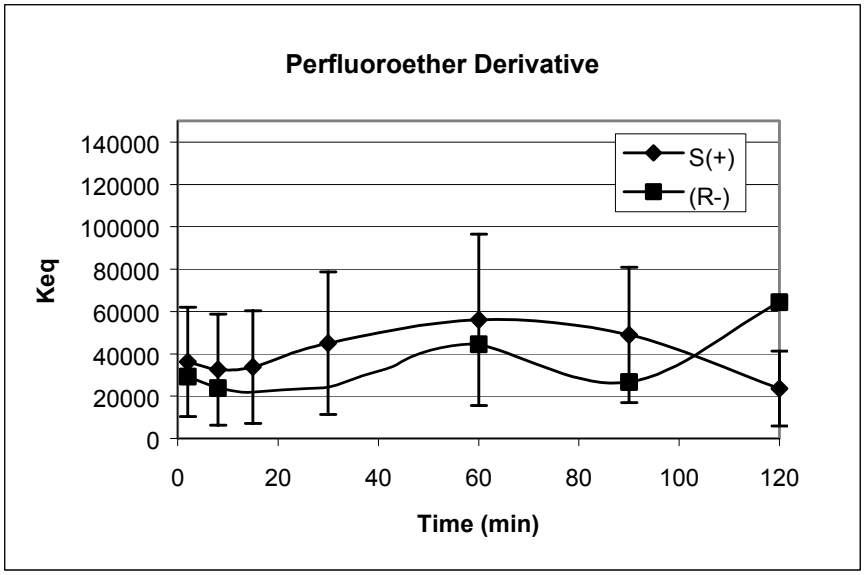

Figure 51 Equilibrium Data for (Perfluoro)-L-Lysine with Alkyl Spacer

In terms of selectivity, slight deviations in the trend lines from the polyperfluoroether (Krytox) analogues are again observed for this family of agents. In Figure 49, a slight preference of the N'-perfluoroether (Lancaster) derivative for the S(+) enantiomer of ibuprofen is observed. This result remained consistent as a function of time. In comparison to its polyperfluoroether (Krytox) analogue, the result is similar except that the polyperfluoroether (Krytox) analogue showed a preference for R(-) enantiomer. A rational explanation for why the polyperfluoroether (Krytox) analogue exhibited a preference for the opposite enantiomer is unknown.

The N-perfluoroether methyl ester (Lancaster) analogue in Figure 50 exhibited no preference for either enantiomer. Interestingly, the third (Lancaster) agent - the perfluoroether agent, exhibited the same cross over effect as observed in Figure 48. However, the switch in enantiomer selectivity occurred with the S(+) enantiomer being dominant in Figure 51 and the

R(-) enantiomer in Figure 48 at the conclusion of the experiments. Experimental error may be one plausible explanation and expanding the number of test sets may reflect a different behavior. Another possible explanation may be attributed to the sensitivity of the conformation of the binding pocket. Synthetic conditions may have altered the configuration of the lysine head group that might impart a slight sensitivity for the R(-) enantiomer. Qualitative polarimetry studies indicated, however, that for the polyperfluoroether (Krytox) derivative, the positive sign of the rotation of light indicated no change in the chiral configuration of the lysine head group.

Molecular modeling studies for this family of agents indicated that no selectivity should be observed for the N-perfluoroether methyl ester and the N'perfluoroether derivatives. The perfluoroether derivative exhibited a slight degree of selectivity, however, the opposite enantiomer was predicted by the theoretical calculations.

8.3.4 The Fluoroalkyl Derivatized Lysine Family

The last set of equilibrium data is presented in Figure 52. This series of resolving agents involved the derivatization of lysine with a fluoroalkyl tail. As stated in Chapter 7, only one out the three agents in this family proved to be soluble within the testing limits of the apparatus. There are some notable observations to make concerning this agent. The first is that equilibrium is readily established within 20 minutes as observed in Figure 52, and this time frame is consistent with the fluoroalkylated quinine and quinidine. A relatively uniform trend line would indicate that the rate of precipitation is significantly slower than the observed reaction equilibrium. Unfortunately, slow precipitation, in terms of a separation scheme, would be

unfavorable due to the long time required for not only precipitation to commence, but also the settling time required to generate a substantial quantity of product.

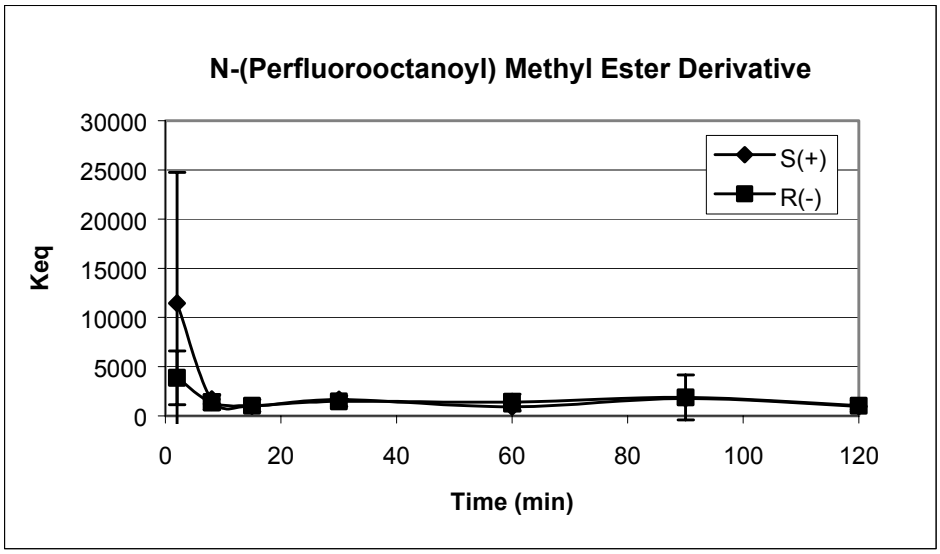

Figure 52 Equilibrium Data for N-(Perfluorooctanoly)-L-Lysine Methyl Ester

Another observation to note is that there appears to be relatively little differentiation between the two enantiomers. Molecular modeling studies indicated a higher degree of selectivity for this agent, with the S(+) enantiomer favored. Lysine, itself, is not a very highly selective agent in CO_2 or in a polar medium as compared to the catalysts used for the asymmetric synthesis of ibuprofen. As reported by Tung and coworkers, the lysine ibuprofenate salts formed in a 95% ethanol/5% H_2O solution form a eutectic point of approximately 55% to 65% of the S(+)-ibuprofenate salt predominating in ethanol. [31] Thus, these salt complexes of the lysine family must be separated as a function of their phase behavior differences alone. The experimental data for all agents presented in this chapter emphasizes that selectivity is highly

dependent upon solvent type. In this instance carbon dioxide does not serve as a conducive medium for these selective reactions, and the resultant ion pairs must exclusively be separated as a function of phase behavior differences.

8.4 Conclusion

A simplified experimental design and equilibrium model were developed in order to evaluate the dominant equilibrium that governs the reaction systems of interest. It was initially assumed that the dominant equilibrium process was the ion pair formation between ibuprofen and the modified resolving agents. From the data presented in this chapter, it was shown that the model used to fit the data did not predict the initial kinetic effects, such as the rates of solubilization, and this was shown as a large initial fluctuation in K_{eq}. Because precipitation was not visually evident, its inclusion into the model was neglected. This simplification fit the data well as long as a stable system was observed. The instability in certain reaction systems, particularly evident with the perfluoroether derivatized agents, failed to be explained by the equilibrium model. Thus, it can be concluded that the model used was too simple in its origins and a more complex model, incorporating multi-equilibria fitted to accurate kinetic parameters is required.

Under the assumption that ion pairing does occur, this experimental design provides a rough comparison to the molecular modeling results. These theoretical calculations assumed that the molecule exists at its optimum bond lengths and angles within a vacuum. Thus, solvent effects, packing forces, and other secondary influences such as intermolecular interactions are

neglected. Table 9 below lists the resolving agent and whether molecular modeling's qualitative prediction was correct. Upon examination of the table, molecular modeling proved to be approximately 60 % on target. In terms of exclusively predicting the selectivity of the agent, though, molecular modeling proves to be an inferior technique due the lack of solvent and solute interaction present in the model. In addition, its usefulness as a comparative method was qualitative at best due to the high experimental errors encountered within the data sets. The utility, then, of molecular modeling for this research project is to serve as a screening tool for candidate agents and it has proven its utility for these purposes.

Table 9 Comparison of Molecular Modeling and Experimental Results

Resolving Agent	Consistent with Molecular Modeling Result
Fluoroalkyl Functionalized Quinine	
Fluoroalkyl Functionalized Quinidine	√
Silicone Functionalized Quinine	
N'-(Polyperfluoroether)-L-Lysine	√ [a]
N-(Polyperfluoroether)-L-Lysine Methyl Ester	√ [a]
(Polyperfluoroether)-L-Lysine with Alkyl Spacer	√ [a]
N'-(Perfluoroether)-L-Lysine	√
N-(Perfluoroether)-L-Lysine Methyl Ester	√
(Perfluoroether)-L-Lysine with Alkyl Spacer	√
N-(Fluoroalkyl)-L-Lysine Methyl Ester	

a) error bars indicate no significant degree of selectivity, while the trend line indicates preference for the opposite enantiomer as predicted by molecular modeling

9.0 ENANTIOMERIC RESOLUTION OF RACEMIC IBUPROFEN

9.1 Design of a Separation System

The goal is to develop a separation system, in which the enantiomers of ibuprofen can be resolved in the form of their diastereomeric complexes. Racemic ibuprofen is soluble in CO_2 at 30 ^{o}C as shown from experimental phase behavior measurements in Figure 53, which is consistent with the results of Khundker and coworkers. [29] Use of the resolving agent in either the unadulterated or functionalized form allows us to examine several types of separation strategies. For example, lysine is essentially insoluble in CO_2 up to the pressure limitations of our instruments, and thus, its native form cannot be used to separate ibuprofen in CO_2. For the case of either quinine or S(+)-phenylglycinol, however, the unaltered resolving agent exhibits measurable solubility in CO_2, but complexes formed from reaction of either of these agents and ibuprofen are essentially insoluble in CO_2, as determined from experimental measurements using the W.B. Robinson Cell. Finally, in the case of lysine or quinine which have been functionalized with fluoro and/or silicone functional groups, both the resolving agent and the resultant complex formed from ibuprofen are soluble in CO_2 under certain conditions of temperature and pressure.

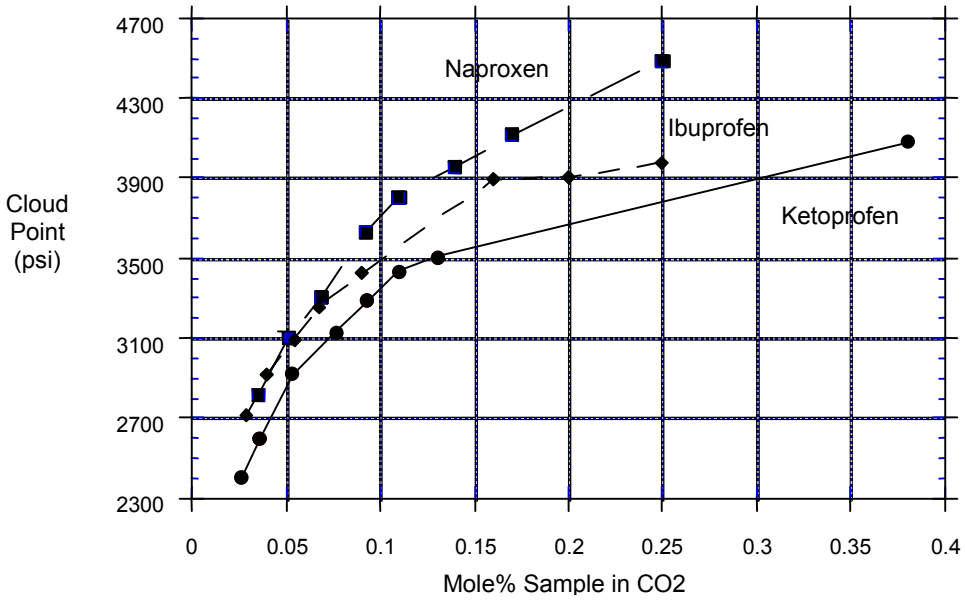

Figure 53 Phase Behavior of Racemic Ibuprofen, Ketoprofen, and Naproxen

9.2 Heterogeneous Reaction Environment

Based upon the phase behavior of the various agents and the diastereomeric complexes formed from them, we have evaluated 3 methods of separation. These methods involve heterogeneous, homogeneous, and homogeneous mixed agent reaction environments. The first method that will be described proposes that if we charge the reactor with, for example unmodified quinine and racemic ibuprofen at conditions where all of the ibuprofen would dissolve, we might expect that as quinine dissolves into solution, diastereomeric salts would form in quantities dependent upon the selectivity of quinine.

One of the primary advantages of performing an enantiomeric separation by this method is that the chiral agent is used in its native form. Thus, the inherent selectivity of the agent is preserved. Preservation of this selectivity, however, is only advantageous if selectivity is not greatly influenced by the solvent type. In this instance, the change in solvent polarity from ethanol to CO_2 is significant. As shown in Chapter 8, any selectivity of the modified resolving agents was absent in the CO_2 medium. Thus, it is anticipated that some decrease in selectivity for the native agents will also be experimentally observed.

Kinetic effects are also expected to contribute to the observed selectivity. The limited solubility of the resolving agent creates a reaction system that is highly dependent upon the rate at which the agent enters into solution. In this manner, the separation becomes a reactive separation which incorporates the elements of selectivity and limited solubility at static operating conditions. [87]

For this set of experiments S(+)-phenylglycinol and quinine were chosen as the test agents for racemic ibuprofen due to the following characteristics for each agent.

- Good selectivity for both of these agents was observed in organic solution. A diastereomeric crystallization was performed in ethanol according to the procedure outlined by Tung and coworkers. [31] The remaining liquor was separated from the solids and analyzed for free ibuprofen content according to the procedure described in Section 8.2. A peak area ratio of approximately 3:1 R(-) to S(+) was obtained for both of these agents.
- These compounds are established resolving agents for racemic acids.

- Both agents exhibited a qualitative degree of solubility in supercritical CO_2 as determined from preliminary phase behavior measurements. The concentration of each agent that was dissolved in solution was estimated at 0.002 mole% and 0.005 mole % for quinine and S(+)-phenylglycinol, respectively.

9.2.1 Experimental

In these experiments, 0.1 mole % of racemic ibuprofen is loaded into a prewarmed high pressure cell, as well as an equimolar amount of (a) S(+)-phenylgylcinol or (b) quinine. The cell is then charged with CO_2 to a pressure of 5800 psi and continually stirred. Samples of the fluid phase are withdrawn at designated time intervals. If formed, precipitate will settle to the bottom of the reactor unit. The base of the reactor was specially machined in the shape of a smooth funnel so that precipitate is directed to the bottom of the reactor.

Samples were analyzed by a Hewlett Packard Series II 1090 HPLC unit using a Chiracil-ODH (Chiral Technologies) chiral column. The mobile phase consists of 98% hexane: 1.9% 2-propanol: 0.1% TFA, and the analysis was run with a flow rate of 0.8 ml/min at 38°C. The run time for sample analysis was 8 minutes. The percent enantiomeric excess (%EE) was calculated as follows

$$\%EE = \frac{\left(\frac{R}{S} - 1\right)}{\left(\frac{R}{S} + 1\right)} x100 \qquad (9\text{-}1)$$

where R is the peak area for the R(-) enantiomer of ibuprofen and S is the peak area for the S(+) enantiomer of ibuprofen.

The results of these experiments for both agents are shown in Figures 54 and 55. S(+)-Phenylglycinol performed poorly, exhibiting only a modest 17% enantiomeric excess after 24 hours in supercritical conditions (T = 35 ^{o}C). No significant enantiomeric excess was evident when the same agent is run in liquid CO_2. These results are contrary to what had been observed in ethanol, and again carbon dioxide does not appear to function as a reaction medium conducive to selectivity for this native agent. Evidence that complexation was occurring was obtained from measuring the concentration of free ibuprofen as a function of time. The reactor was loaded with an initial charge of 0.1 mole % of ibuprofen. After 30 minutes, the concentration of free ibuprofen was reduced to 0.006%. At the conclusion of the experiment greater than 99% of the ibuprofen was no longer available in its free acid form.

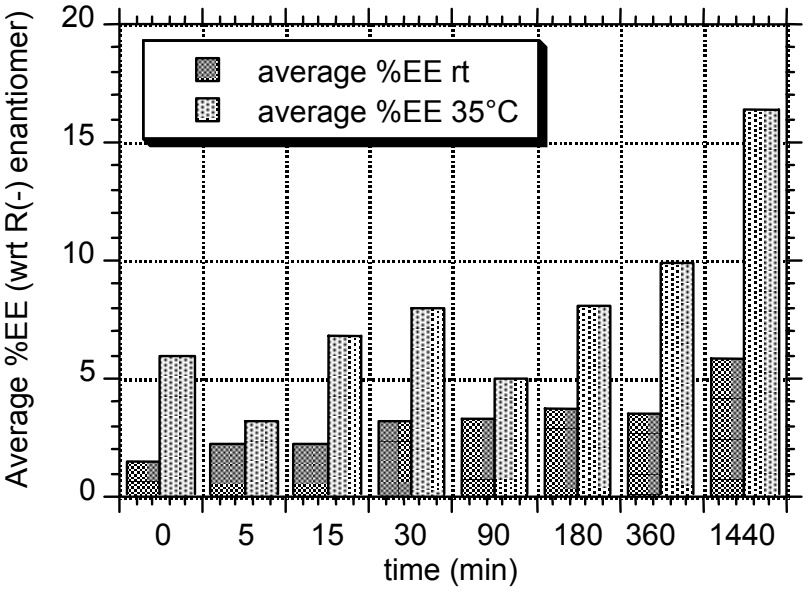

Figure 54 % Enantiomeric Excess: S(+)-Phenylglycinol as Resolving Agent (rt = 25 ^{o}C)

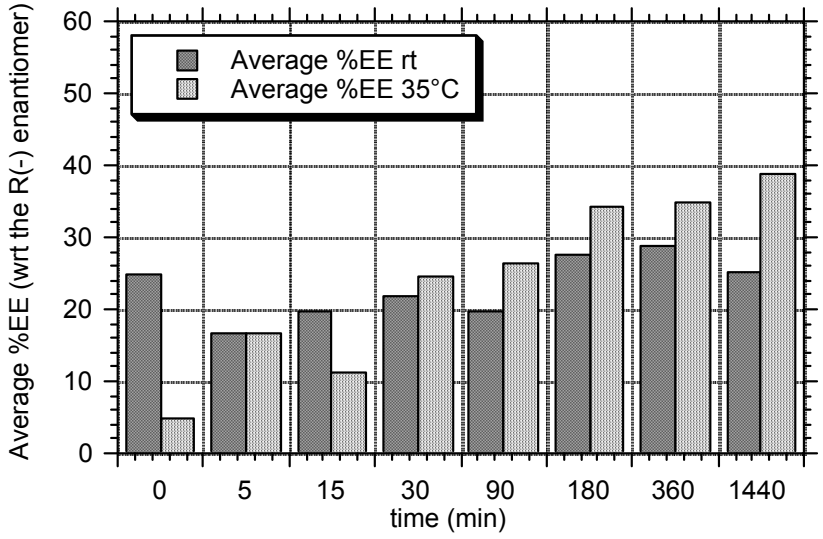

Figure 55 % Enantiomeric Excess: Quinine as Resolving Agent (rt = 22 ^{o}C)

If the agent's selectivity was truly a dominant parameter for this type of separation, then it is expected that the highest enantiomeric excess would be observed in the initial phase of the experiment. This type of separation would mimic a kinetic resolution. Instead, the highest % EE is observed towards the conclusion of the experiment at t = 24 hours (1440 min). These observations lead to the conclusion that the selectivity of S(+)-phenylglycinol is severely depressed in a carbon dioxide environment.

In addition, the measurement of higher %EE at longer reaction times also gives an indication that the %EE is a result of solubility differences between the formed complexed salts. Precipitation was not visually observed within the first 6 hours, but lack of a visual observation does not necessarily preclude any precipitation that might have occurred within the 24 hour time period. Samples of the fluid phase only are taken and are filtered in line prior to loading in the sampling value. The fact that 99% of the ibuprofen was bound indicates that complexation did occur and whether the complexes precipitated out, were soluble at their specific concentration, or were simply dispersed in the fluid phase by the action of the stirrer is unknown.

Quinine exhibited a measured 39% enantiomeric excess under the same operating conditions, which is comparable to the measured 58 % EE in organic solution, and is a marked improvement over the results obtained for S(+)-phenylglycinol. In addition, this particular agent appeared less sensitive to differences between liquid and supercritical conditions. Similar to S(+)-phenylglycinol, greater than 90% of the free acid form of ibuprofen was bound by the conclusion of the experiment. However, a concentration of 0.006 mole % ibuprofen in CO_2 was not realized until 180 minutes versus 30 minutes for S(+)-phenylglycinol. This result would indicate that either quinine does not enter the bulk fluid phase at a rate similar to S(+)-phenylglycinol, the reaction rate for quinine and ibuprofen is much slower than S(+)-

phenylglycinol, side reactions are more predominant with the quinine agent system, or a combination of all these factors. As discussed previously for S(+)-phenylglycinol, larger enantiomeric excesses were observed at longer reaction times again indicating a depression of selectivity and % EE being predominantly a function of variable phase differences between the two salts.

These two agents were also chosen to qualitatively compare the difference between the efficiency of binding of a primary and tertiary amine. A comparison of these two agents gives insight into the effects of potential carbamate formation at operating conditions. From the results reported above, each agent was capable of binding almost the complete stoichiometric ratio of ibuprofen, indicating that carbamate formation did not occur or did not preclude binding of the chiral agent to ibuprofen. The worst case scenario would be reaction of CO_2 with a primary amine. Either the primary amine of S(+)-phenylglycinol does not bind with CO_2 or ibuprofen is able to displace the CO_2 group.

Because quinine exhibited reasonable enantiomeric excess at a 1:1 mole ratio with racemic ibuprofen, it was hypothesized that a 1:2 mole ratio of agent to ibuprofen would yield improved enantiomeric excess if selectivity were truly a dominant parameter in this type of separation scheme. Ideally in crystallization, if only one of the salts crystallizes out of solution, then one half of the mole equivalent is required due to large differences in solubility of bound and unbound species if selectivity exists. [5] As shown in Figure 56, the enantiomeric excess under the same test conditions was decreased. This result supports the assertion from the previous experiments that agent selectivity for the unadulterated agents is severely attenuated and separation must primarily occur by phase behavior differences alone.

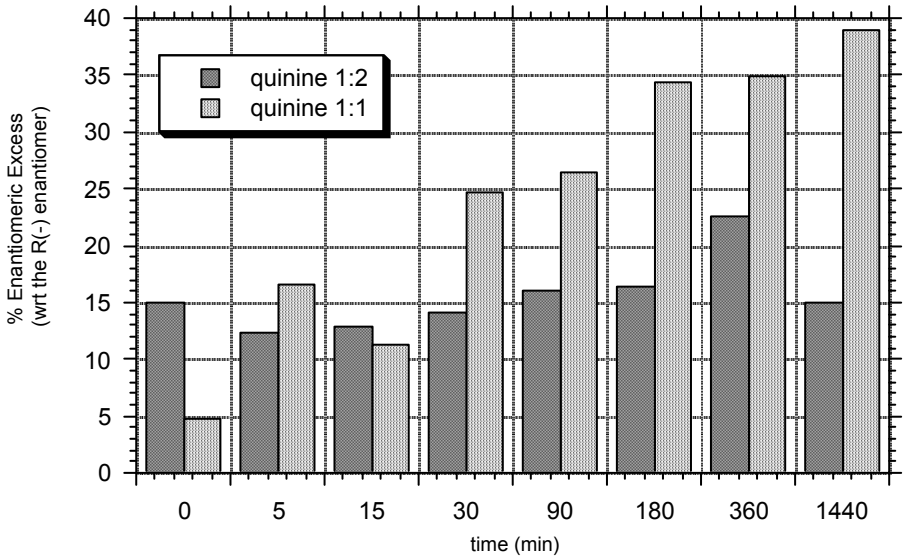

Figure 56 % Enantiomeric Excess: Comparison of 1:1 and 1:2 Mole Ratio of Quinine to Ibuprofen

9.2.2 Concluding Remarks

Though this mode of resolution for the enantiomers of ibuprofen offers many process advantages, its functionality as a separation system is poor. This poor performance is mainly due to the inability of the resolving agents in supercritical CO_2 to effectively discriminate between the two enantiomers of ibuprofen. The problem is also compounded by the poor solubility of the resolving agent, giving rise to other dominant kinetic effects. The introduction of a polar modifier, such as methanol, could potentially aid in the solubilization of the agent and its resultant salt complexes. This concept was effectively demonstrated by Eckert and coworkers. They formed two diastereomeric salts, (R)-(+)-α-phenethylammonium (R)-(-)-mandalate and (R)-(+)-α-phenethylammonium (S)-(+)-mandalate using (R)-(+)-α-phenethylamine as the chiral

discriminator in polar organic media. Interestingly, CO_2 was added to the organic phase as an anti-solvent to precipitate one of the salts. They were able to obtain %EE in excess of 90%. [88]

The advantages of using a modifier in this scenario are 1) the potential increase in the rate of agent solubilization 2) the potential improvement in agent selectivity and 3) the enhancement of solubility differences between the diastereomeric salt pair. These factors lead to a separation system more similar to the traditional crystallization schemes. The drawback to using a modifier, however, is the introduction of an organic component to an organic free solvent/reaction medium. These issues have led to a redesign of the resolving agent, which has resulted in the development of a homogeneous phase separation system. Generation of a homogeneous mixture that includes salts, i.e. the reaction products of resolving agents plus ibuprofen, requires that we derivatize the resolving agents to enhance their solubility and thus that of the salts in CO_2. Consequently, we have functionalized those resolving agents under consideration with so called "CO_2-philic" moieties (or "tails"). This is the chemical equivalent of a bound co-solvent to enhance solubility.

9.3 Homogeneous Reaction Environment

In the previous section we described the results of experiments where a heterogeneous system (solution plus residual solid) was employed in an attempt to separate the enantiomers of ibuprofen in CO_2. By contrast, in this section we describe the results of a separation performed from an initially homogeneous mixture of ibuprofen, resolving agent, diastereomeric salt, and CO_2. As described in Chapter 8, the ability to separate the enantiomers of ibuprofen as a

function of the agent's natural selectivity was not evident. The data experimentally measured in Chapter 8 is supported by the inability to obtain high %EE from the previous heterogeneous reaction system. Thus, the separation of the enantiomers of ibuprofen must be performed strictly as a function of phase behavior differences alone.

9.3.1 Resolving Agent Selection

Whether a polar modifier or actual covalent modification of the resolving agent is the method of choice to create a homogeneous reaction/separation medium, the separation methodology is based upon a thermodynamic resolution. As discussed previously, equilibrium conditions are established within a relatively short reaction time. The reaction time is rapid enough so as to make a kinetic resolution improbable, especially with the current design of the operating system.

Based upon the molecular modeling results presented in Chapter 4 and the measured equilibrium constants presented in Chapter 8, the fluoroalkylated quinine and lysine agents as shown in Figure 57 were selected for this series of experiments. As described previously, use of either the perfluoro, polyperfluoro, or silicone modified agents wasn't deemed sensible based upon the inherently high solubility of these compounds and their complexes, resulting in a more difficult separation. Because the salt complexes are to be separated as a function of phase behavior alone, the fluoroalkylated agents have the advantage of poorer solubility.

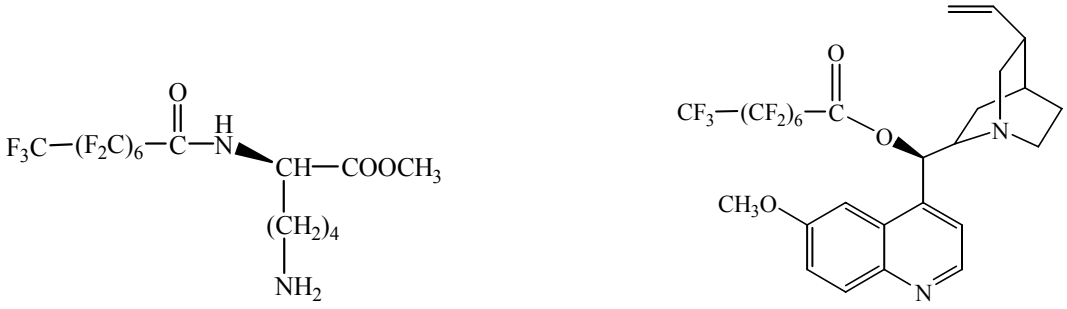

Figure 57 Fluoroalkylated Quinine and L-Lysine Methyl Ester

It was hypothesized that the salts formed from the complexation of ibuprofen and modified resolving agent might exhibit phase behavior patterns which are different from one another, and that those phase behaviors would be different from free ibuprofen and free resolving agent. This was experimentally verified as shown in Figures 58 and 59. Expanded views of the phase behavior of the individual salts are shown in Figures 60 and 61. The data in Figures 58 and 59 suggest that one can certainly separate the ibuprofenate salts from residual resolving agent and from free ibuprofen, but that a clean separation of the two salts may be challenging based on the fact that the maximum observable difference in solubility between the two salts is approximately 150 psi for the L-lysine based agent and 300 psi for quinine based agent.

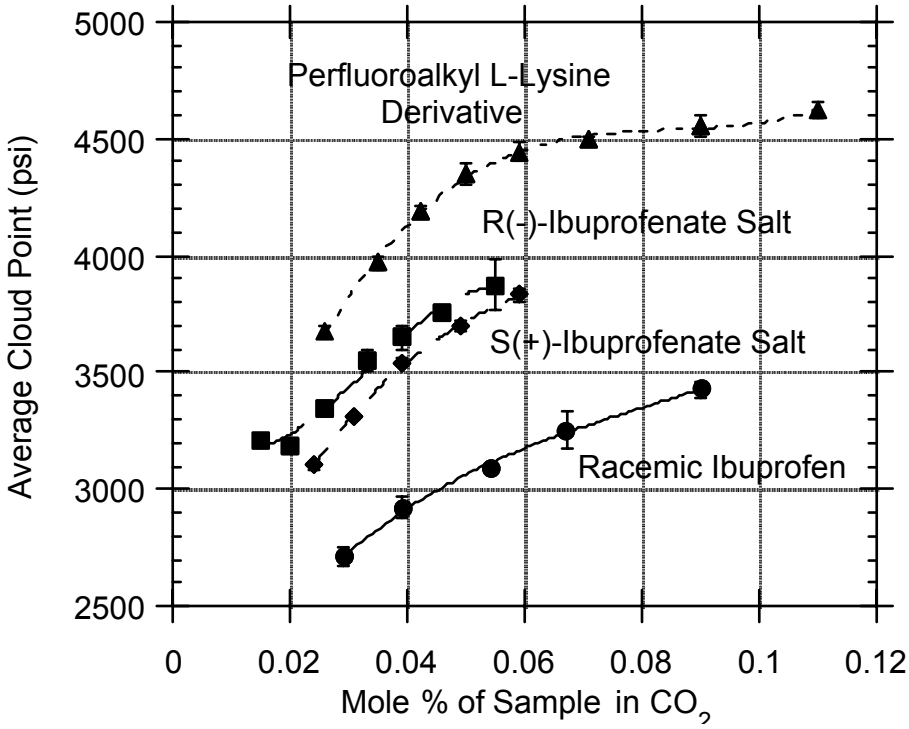

Figure 58 Phase Behavior Diagram for Fluoroalkyl L-Lysine Resolving Agent, Racemic Ibuprofen, and the Corresponding Ibuprofenate Salts

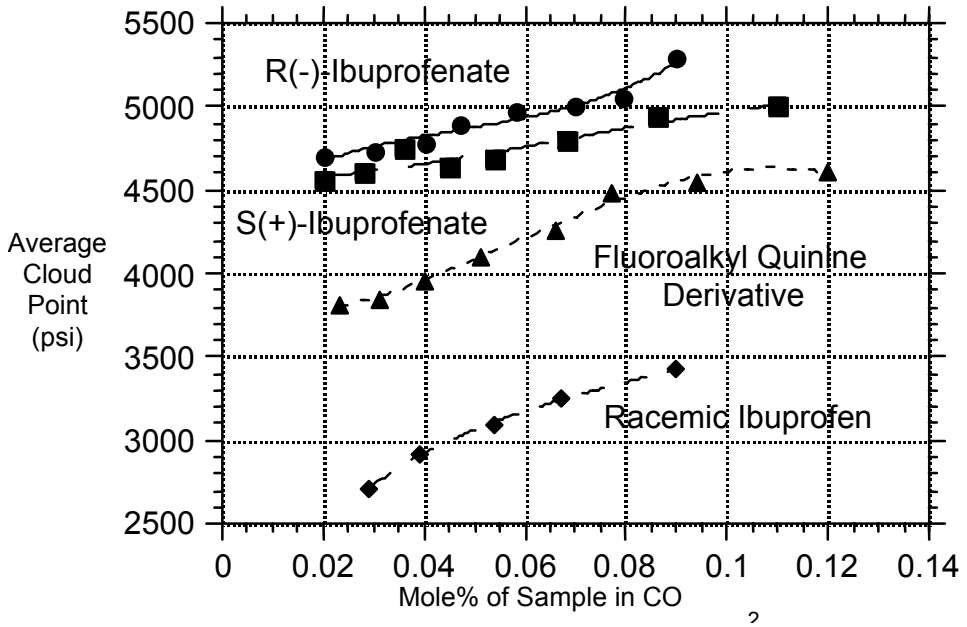

Figure 59 Phase Behavior Diagram for Fluoroalkyl Quinine Resolving Agent, Racemic Ibuprofen, and the Corresponding Ibuprofenate Salt

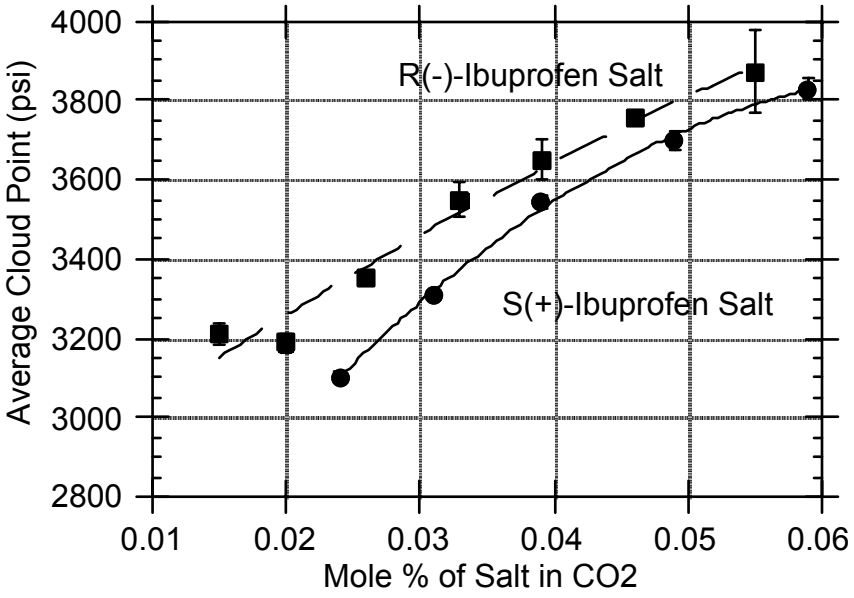

Figure 60 Phase Behavior Diagram for Fluoroalkyl L-Lysinate Salts

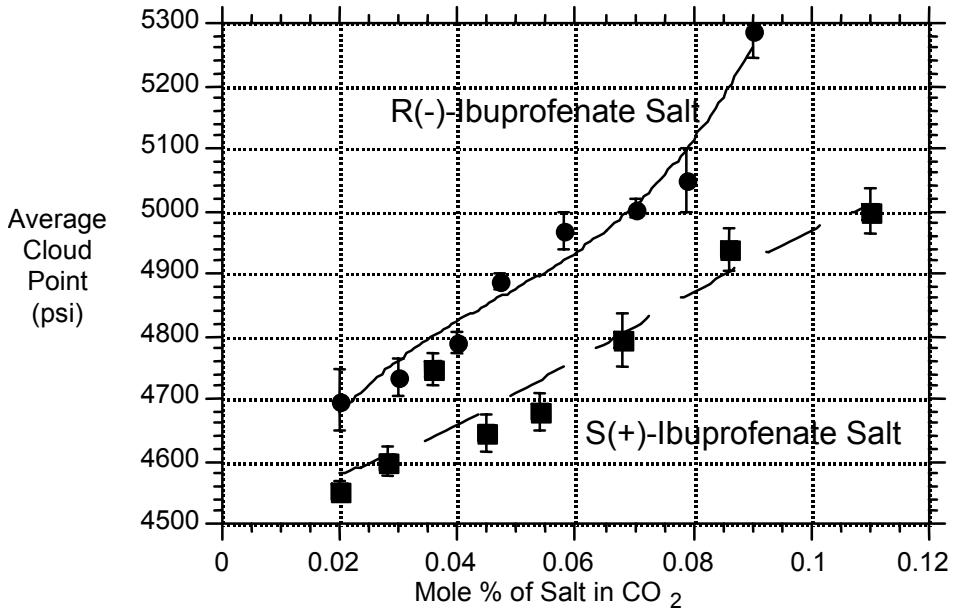

Figure 61 Phase Behavior Diagram for Fluoroalkyl Quinine Ibuprofenate Salts

9.3.2 Experimental Procedure

Now that the phase behavior of the salt complexes for the two different resolving agents have been verified, experiments were conducted in order to assess an optimal starting concentration of the reactants in order to obtain a maximum separation. Examination of Figures 60 and 61 show that the phase behavior differential along the measured concentration ranges remained relatively consistent for the lysine based agent and slightly higher differentials at increasing salt concentrations for the quinine based agent. It is expected then that the starting concentration of lysine based reagents should be independent of concentration, while variability would be expected with the quinine based reagents.

These particular experiments were carried out at two different temperatures, namely 26°C and 35°C in order to examine any difference in employing a sub or supercritical reaction system. Typically, a 1:1 mole ratio of the resolving agent to ibuprofen is loaded into the high pressure cell, which is then charged with CO_2 to a pressure of 5800 psi. This pressure is above the phase boundary curves of each of the components, as shown in Figures 58 and 59. In order to determine the optimal initial concentration, the pressure is lowered suddenly by 800 psi to 5000 psi, the contents of the cell allowed to settle, and the solution sampled for the free and bound ibuprofen in the fluid phase using the reactor configuration as shown in Figure 32. This procedure is repeated three times for each concentration evaluated.

Once determining an optimal initial reactant concentration, fractionation experiments were performed. These experiments comprised three cuts per experiment in order to determine if the ability to obtain higher %EE as a function of subsequent precipitation steps was obtainable. It was initially anticipated that the number of cuts required would be large due to the similarity in phase behavior between the diastereomeric salts. The fractionation process was setup using the reactor configuration as shown in Figure 33.

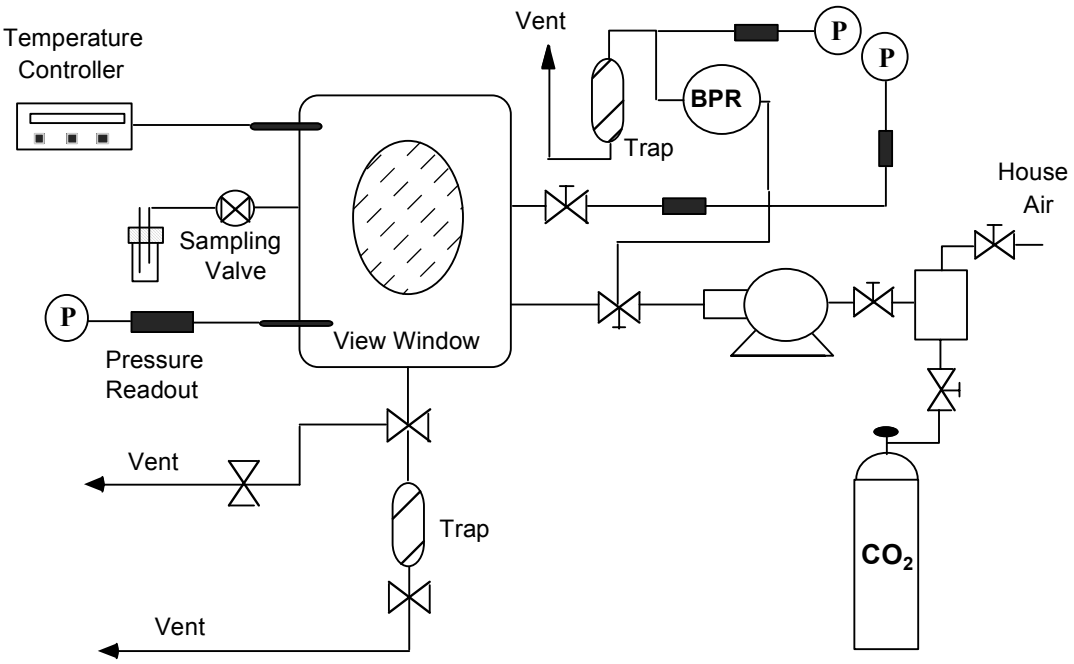

Figure 33 Modified High Pressure Batch Reactor

Typically for the fractionation process, the reactor is initially loaded with a 1:1 mole ratio of resolving agent to racemic ibuprofen to attain a concentration range of 0.025 to 0.06 mole %. The reactor is then charged with CO_2, pressurized to 5800 psi, and stirred for 60 minutes. The pressure is lowered via the sampling valve by a designated increment, typically 800 psi, and any precipitated material is allowed to settle for 30 minutes. Material which remains soluble in CO_2 after the pressure quench is collected by flushing the reactor with pure CO_2 (at the quench pressure) through the back pressure regulator and into a trap (Thar Designs) where the pressure has been lowered to 1200 psi. The reactor is isolated, and the flush line is further washed for an additional 5 minutes. The product from the trap is collected, weighed, and analyzed. The reactor is then re-pressurized to the initial reaction pressure and stirred for 30 minutes. The procedure of

depressurization, settling, wash, and re-pressurization is repeated two more times to constitute an experimental test set.

Samples were analyzed by a Hewlett Packard Series II 1090 HPLC unit using a Chiracil-ODH (Chiral Technologies) chiral column. The mobile phase consists of 98% hexane: 1.9% 2-propanol: 0.1% TFA, and the analysis was run with a flow rate of 0.8 ml/min at 38°C. The run time for sample analysis was 8 minutes. The percent enantiomeric excess (%EE) was calculated as follows

$$\%EE = \frac{\left(\frac{R}{S} - 1\right)}{\left(\frac{R}{S} + 1\right)} x100 \tag{9-2}$$

where R is the peak area for the R(-) enantiomer of ibuprofen and S is the peak area for the S(+) enantiomer of ibuprofen.

9.3.3 Results and Discussion

The results using the fluoroalkylated quinine resolving agent are shown in Table 10. Three initial starting concentrations were evaluated based upon the least dilute to the most dilute according the concentration profile in Figure 61. Results indicate that the more the dilute the solution, the higher the measured enantiomeric excess. This is surprising, as the phase behavior in Figure 61 shows that the pressure differential between the phase boundary curves of the two salts broadens as the solution becomes more concentrated. This result gives an indication that the collective phase behavior of the solution is not well represented by the phase behavior curves

generated independently for these salts. In addition, the results for liquid CO_2 are significantly poorer than for supercritical CO_2 as was also observed for the heterogeneous reaction system. A smaller phase behavior differential for the salts at the lower temperature is assumed responsible for the measured decrease in enantiomeric excess. Increasing the temperature is often an effective means to improve the phase behavior differential between two components.

Based upon these preliminary results, fractionations were performed at 35^{o}C and at an initial reactant concentration of 0.02 mole %. The results of these tests are also shown in Table 10. The enantiomeric excess measured in each fractionation remained relatively consistent. This result indicates that there is no significant dilution of the reaction products after fractionation. From the trap, approximately 10% of the original ibuprofen concentration was collected, which is consistent with the phase behavior for these salts. Most material is expected to precipitate out due to a modest difference in solubility between the two salts.

Table 10 Fractionation Results for the Fluoroalkylated Quinine Resolving Agent

DETERMINATION OF STARTING CONCENTRATION		
Mole %	**% Enantiomeric Excess**	
	35 °C	**25 °C**
0.05	**15.9 ± 6.9**	**0**
0.03	**30.5 ± 12.7**	**7.28 ± 1**
0.02	**46.0 ± 9.0**	**3.50 ± 1**
FRACTIONATION RESULTS		
Fraction	**% Enantiomeric Excess**	
	35 °C	
1	**44.1 ± 8.3**	
2	**52.8 ± 22.2**	
3	**45.8 ± 12.8**	

The results for the fractionations performed with the fluoroalkylated L-lysine derivative are presented in Table 11. Again, three concentration ranges were measured to determine the optimal starting concentration. However, the most dilute concentration tested, 0.016 mole %, resulted in no detectable ibuprofen (within the UV detector limits of the HPLC). Enantiomeric excess at the higher initial concentrations was slightly below that obtained with the fluoroalkylated quinine agent and was verified by separations. Comparing the phase behavior of the two salts in Figures 60 and 61, the lysinate salts exhibited a smaller solubility differential

than the quinine based salt complexes. Therefore, smaller observed enantiomeric excess is not an entirely unexpected result.

Fractionations were performed at 35^{o}C and at an initial reactant concentration of 0.03 mole %. At 0.03 mole % enantiomeric excesses of at least 30% would be expected, however, % EEs over 20% were not realized. According to Figure 60, a maximum solubility difference of 150 psi between the salt complexes was measured. With the current equipment design, a fine separation of 150 psi is improbable. Interestingly, unlike the fluoroalkylated quinine agent whose enantiomeric excess is predictable from its phase behavior, the fluoroalkylated L-lysine produced an opposite result. According to its phase behavior diagram, an enantiomeric excess with respect to the R(-) enantiomer of ibuprofen should have been observed since the R(-) enantiomer is the more soluble salt. Instead, an enantiomeric excess in relation to the S(+) enantiomer was measured and is consistent with the molecular modeling predictions from Chapter 4 and the work of Tung and coworkers. [31]

The phase behavior measurements for these salts were performed at room temperature. By raising the temperature to 35 °C, the solubility order of the salts may have been potentially inverted. This effect has been documented with ibuprofen and L-lysine in a diastereomeric crystallization process. At higher temperatures, the R(-) enantiomer, as a lysinate complex, and not the S(+) enantiomer preferentially precipitates from solution. [32]

Table 11 Fractionation Results for the Fluoroalkylated L-Lysine Resolving Agent

DETERMINATION OF STARTING CONCENTRATION		
Mole %	**% Enantiomeric Excess**	
	35 °C	**25 °C**
0.06	**30.0 ± 2.7**	**8.6 ± 1**
0.03	**34.0 ± 12.7**	**6.70 ± 1**
0.016	**N/A**	**N/A**
FRACTIONATION RESULTS		
Fraction	**% Enantiomeric Excess**	
	35 °C	
1	**19.8 ± 12.2**	
2	**9.6 ± 2.4**	
3	**21.4 ± 12.7**	

9.3.4 Concluding Remarks

This series of experiments has shown that measurable enantiomeric excess may be obtained by complexation of a chiral agent to the enantiomers in a racemic mixture with direct separation of the resultant salts. The disadvantage of this method, however, is the poor yield. In order to optimize the separation process, then, yield must be improved while maintaining a desirable enantiomeric excess. In order to realize that goal, the phase behavior of the resultant salts must differ substantially. It is hypothesized that diastereomeric salts formed from the same

chiral agent will not give the desired phase behavior difference required for better yield in a CO_2 environment. Instead, salt complexes formed from at least two chiral agents, whose solubility varies considerably, may produce the desired outcome. The next section of this thesis briefly describes a preliminary investigation of multi-agent separations.

9.4 Mixed Agent Reaction Environment

The use of a single agent and pressure variations to resolve racemic ibuprofen produced preliminary results for %EE that are promising, yet the yields per fractionation were low. This is primarily because the phase boundary curves for each diastereomeric salt are quite close to each other in P-x space. When examining the phase behavior and selectivity of the various functionalized resolving agents, however, we observed that it might be possible to achieve both greater purity and higher yield through the use of a mixed agent system.

Unlike the previous method of separation, which was based on the resultant phase behavior of the salts alone, this mode of separation is surmised to be dependent upon not only the phase behavior of the salts, but also any limited competitive binding by the resolving agents. It is hypothesized that one agent will exhibit a small, preferential affinity for one enantiomer, leaving the complimentary agent the remainder of the ibuprofen to bind. In addition to selectivity if any exists, the phase behaviors of the resultant salts must differ appreciably in order to efficiently separate the two enantiomers. This method, in theory, would prove advantageous over the use of single agent resolutions as discussed in the previous section.

9.4.1 Results and Discussion

The following resolving agents as shown in Figure 62 were chosen for a mixed agent enantiomeric separation based upon the large difference in solubility between them (> 1000 psi) and their natural, albeit minimal specificity for the enantiomers of ibuprofen as shown in Tables 7a-7d and in Chapter 8.

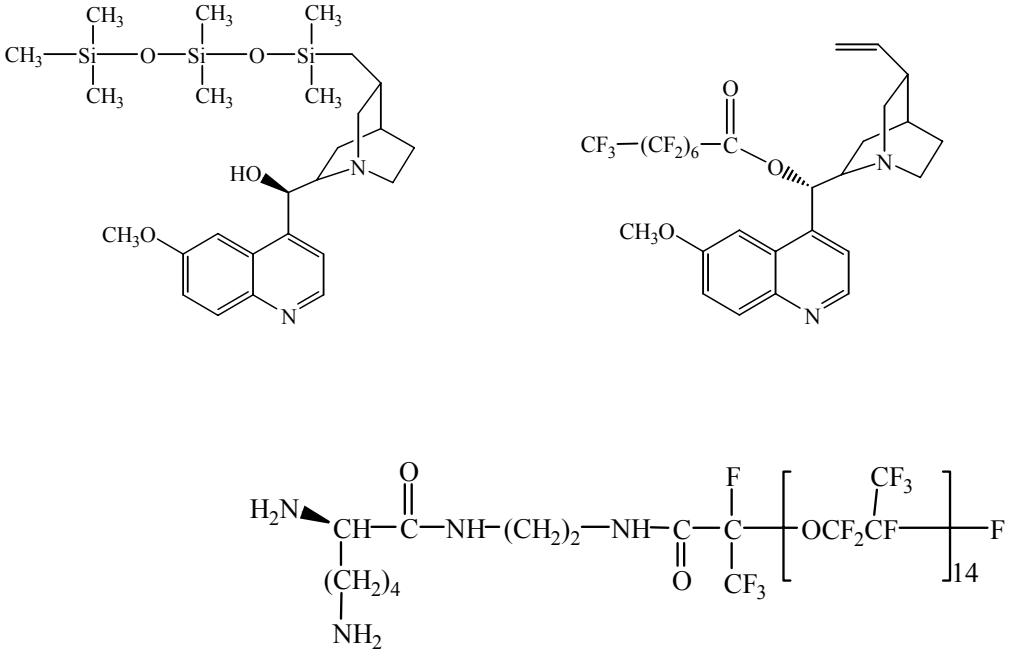

Figure 62 Derivatives Chosen for Mixed Agent Separation

The silicone modified quinine derivative was chosen because it retained the greatest theoretical and apparent selectivity in carbon dioxide of all agents tested. Polyhexafluoropropylene oxide L-lysine modified at the alpha carboxy group was chosen as a control molecule because it showed no preferential selectivity to either ibuprofen enantiomer.

Each of these two molecules were paired with a fluoroalkylated quinidine due to its apparent reduced preference for S(+) enantiomer of ibuprofen. The same experimental procedures described in Section 9.3.2 for use with the single agents were followed, except that a 0.5 mole ratio of each resolving agent to ibuprofen was used. The results for this series of experiments are shown in Figure 63. The percent enantiomeric excess is reported in Figure 63 relative to the S(+) enantiomer.

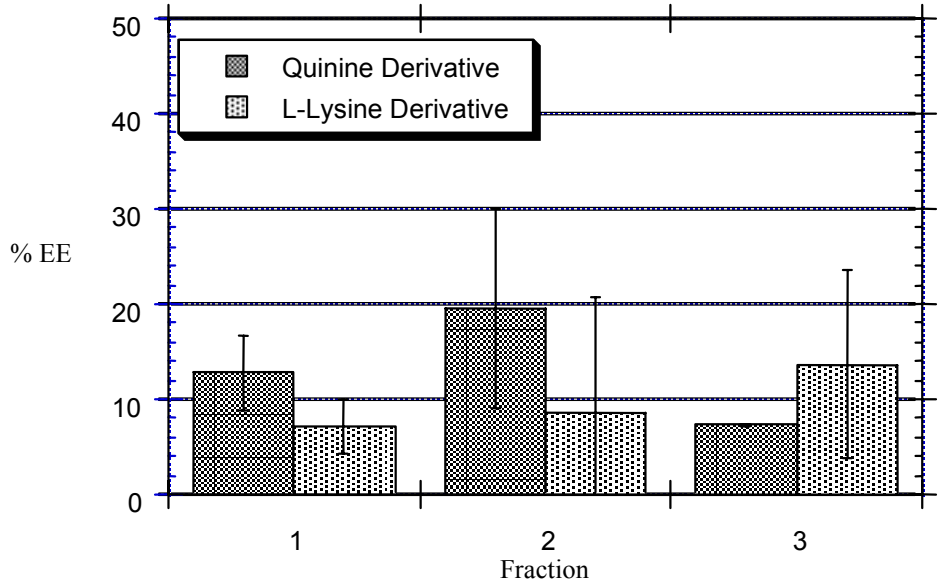

Figure 63 Enantiomeric Excess Obtained for the Silicone Derivatized Quinine/Fluoroalkylated Quinidine and the Polyhexafluoropropylene oxide L-lysine/Fluoroalkylated Quinidine Mixed Agent Systems

The maximum yield for either resolving agent pair reached no greater than 20 % EE. Plausible explanations for this observed decreased in enantiomeric excess are outlined below.

- All of the modified agents exhibited no selective behavior. Thus, the anticipated competitive binding of ibuprofen did not occur.
- The collective phase behavior of the mixed agent system does not exhibit the same or similar phase behavior distinctly associated with each individual species as shown in Chapter 7. At equilibrium there would exist at least five species, two sets of salt complexes and CO_2, assuming that all of the ibuprofen was bound. The more soluble salts of the diastereomeric salt pairs present greater difficulty in separation, because their phase behavior is not dictated by a contribution of the head and tail group. Instead, the phase behavior is governed primarily by the tail alone.
- Typical of diastereomeric salts, the formation of an unfavorable eutectic is possible.
- Polyhexafluoropropylene oxide modified agent may promote a slight cosolvent effect. Empirical demonstrations in lab have shown the solubility of materials to be greatly enhanced when operating equipment is contaminated with this material.

Performing a mass balance on ibuprofen, it was evident that the goal of improved yield was reached. Up to 65% of the more soluble resolving agent was recovered from the trap as compared to 10% with the single agent system. However, this occurred at the expense of decreased enantiomeric excess.

9.4.2 Concluding Remarks

The mixed agent system presents the most viable approach to achieving good enantiomeric separations via diastereomeric ion pairing in supercritical carbon dioxide. Unfortunately, the most theoretically selective of the resolving agents, in this case, is also the most difficult to separate due to its high solubility in CO_2. What resulted was a separation of almost one half of the 50:50 mixture. A highly selective agent is critical if this design is to be efficient. In order to utilize a mixed agent system for fine separations, not only are less CO_2 "philic" substitutes are required, but also specialized agents capable of retaining selectivity in carbon dioxide.

10.0 RECOMMENDED FUTURE WORK

The work, which has been presented in this thesis, provides fundamental information towards the development of a fine separation system for enantiomers as a function of their diastereomeric salts. Preliminary fractionations indicate that separations performed in this manner result in moderate enantiomeric excess, but poor yield. In order to increase not only the enantiomeric excess of the product but also recovery, an investigation into the nature of the resolving agent is required.

The inherent drawback to the currently designed resolving agents is the fact that the solubility of the diastereomeric salts produced from the reaction of ibuprofen and chiral agent are largely governed by the solubility of the "CO_2 philic" chain of the resolving agent. Compounding the difficulty in separation is also the fact that the individual salts do not exhibit independent phase behaviors in solution. Rather they form eutectics, and the position of that particular eutectic dictates the maximum separation that can be achieved. In order to achieve the desired separation, then, new research into chiral binding should be explored. This thesis presents data which indicates that a non-chromatographic, non organic medium separation may be plausible. The key is to improve upon the selectivity of the agent. Potential avenues of resolving agent modification are outlined below.

Resolving Agent Design

Affinity Modulation

Affinity modulation is a term to describe the development of a three party binding system for the purpose of enhancing the affinity of a ligand to its target molecule. The technique was developed by Gerald Crabtree and Thomas Wandless at the Howard Hughs Medical Institute. The basic premise behind this technique is to increase (or is some cases decrease) the affinity of a ligand for its target molecule by "borrowing" a third specific binding surface. [73]

What would be required is actually an additional chiral bridge from ibuprofen to the native resolving agent. The orientation of the chiral alcohol of ibuprofen and of a hydrogen bonding functionality of the resolving agent would define the ability of the third chiral bridge to form or not to form a ternary complex. Considering some of the primary factors which influence phase behavior, which include molecular weight and polarity, the chiral bridge could be designed in such a way as to either produce a significant increase or decrease in CO_2 solubility upon complex formation.

Inorganic Selective Binding Agents

The preferred route for the production of single isomer pharmaceuticals is chemical assymetric synthesis. The primary advantage of this production route is that theoretically no waste, i.e. unwanted isomer, is produced and this presents a cost effective strategy for single isomer drug development. The lesson learned from this form of pharmaceutical development is that there exists a growing pool of highly specific inorganic catalysts. For example, in order to produce a compound with 99% EE, the catalyst used must exhibit a 200:1 preference for one enantiomer. In a supercritical CO_2 extraction scheme, preferential binding to one enantiomer

may potentially impart large solubility differences, attributable to both a difference in molecular weight and solute- CO_2 interactions. This would allow for the extraction of one unbound enantiomer, since ibuprofen is a CO_2 soluble material. The catalyst in this case would not be utilized as a viable component in a reaction scenario, but rather as a means to selectively adjust the physical parameters of one of the enantiomers of ibuprofen in its racemic mixture.

Molecular Modeling

A key component in the investigation of determining the optimal resolving agent for a supercritical CO_2 extraction/fractionation system is the use of molecular modeling. This technique serves a twofold purpose. First, it functions as a laboratory time saving technique by allowing the investigator to construct and complex molecules. As shown in this thesis, structure is a predominant factor in a molecule's ability to dissolve in CO_2. Strain energy and geometrical evaluation are useful tools to screen individual candidates.

An in depth calculational design is required in order to construct a highly selective agent. This includes not only modeling the agent in vacu, but also in a CO_2 solvent box. Because CO_2 –solute interactions govern solubility, a more precise theoretical model of how these agents and ibuprofen would behave in response to each other is vital. The key to efficiently separating these enantiomers from their racemic mixture in a supercitical carbon dioxide medium is phase behavior. The more information which can be derived about CO_2 and structure relationships, the more successful a resolving agent system may be chosen and applied.

The second function of molecular modeling is its utility as a method for theoretical model development. Predicting the solubility of molecules in carbon dioxide is an area of research, which has been in progress for more than twenty years. In addition, the force fields, which are

used for calculational design, are consistently improving and incorporating more accurate property descriptions of carbon dioxide. Developing a theoretical model for enantiomeric resolutions of the nature reported in this thesis is not only innovative, but would provide valuable information in relation to alternative means of racemic resolution in non-organic media.

Reactor Design

Ideally, the standard batch reactor is useful for fractionation experiments. In practice, however, there is room for improvement in the current fractionation high pressure setup. Areas of improvement include better control of the downstream vent for collection of dissolved materials. The maximum amount of total product recovery with the current reactor system was approximately 63%. Material was potentially lost in deposition in the lines and through the downstream trap vent line. This includes redesign of the collection trap and tighter control of the vent pressure through the addition of a back pressure regulator.

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